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ABSTRACT

Material on the membrane filter methods and the most probable number method for determining bacteriological contaminants listed in the interim primary drinking water regulations is presented. This course is for bacteriologists and technicians with little or no experience in bacteriological procedures required to monitor drinking water, though students should have basic skills used in bacteriology laboratory operations. Chapters include reading materials, laboratory activities, and some related references. (CO)

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United States
Environmental Protection
Agency

National Training
and Operational
Technology Center
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Water

Methods for the Determination of Bacteriological Contaminants in Drinking Water

Training Manual

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August 1979
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METHODS FOR THE DETERMINATION OF BACTERIOLOGICAL CONTAMINANTS IN DRINKING WATER

This student manual was developed by the U.S. Environmental Protection Agency, National Training & Operational Technology Center with the Technical Support Division in response to a request from the Office of Drinking Water.

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

DISCLAIMER

Reference to commercial products, trade names, or manufacturers is for purposes of example and illustration. Such references do not constitute endorsement by the Office of Water Program Operations, U.S. Environmental Protection Agency.

This manual has been prepared from the National Interim Primary Drinking Water Regulations and the references contained therein which constitute the legal authority for these procedures. When used within a State having been granted primary enforcement authority, that State's regulations will then constitute the legal authority and should be followed.

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PART IV:

ENVIRONMENTAL PROTECTION AGENCY

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WATER PROGRAMS

National Interim Primary Drinking Water Regulations

Title 40—Protection of Environment

CHAPTER I—ENVIRONMENTAL PROTECTION AGENCY

SUBCHAPTER D—WATER PROGRAMS

[FRL 464-7]

PART 141—NATIONAL INTERIM PRIMARY DRINKING WATER REGULATIONS

On March 14, 1975, the Environmental Protection Agency (EPA) proposed National Interim Primary Drinking Water Regulations pursuant to sections 1412, 1414, 1415, and 1450 of the Public Health Service Act ("the Act"), as amended by the Safe Drinking Water Act ("SDWA," Pub. L. 93-523), 40 FR 11990. EPA held public hearings on the proposed regulations in Boston, Chicago, San Francisco, and Washington during the month of April. Several thousand pages of comments on the proposed regulations were received and evaluated. In addition, the Agency has received comments and information on the proposed regulations from the National Drinking Water Advisory Council, the Secretary of Health, Education, and Welfare, and from numerous others during meetings with representatives of State agencies, public interest groups and others.

The regulations deal only with the basic legal requirements. Descriptive material will be provided in a guidance manual for use by public water systems and the States.

The purpose of this preamble to the final regulations is to summarize the most significant changes made in the proposed regulations as a result of comments received and the further consideration of available information. A more detailed discussion of the comments and of changes in the proposed regulations is attached as Appendix A.

WATER SYSTEMS COVERED

The Safe Drinking Water Act applies to each "public water system," which is defined in Section 1401(4) of the Act as "a system for the provision to the public of piped water for human consumption, if such system has at least fifteen service connections or regularly serves at least twenty-five individuals." Privately owned as well as publicly owned systems are covered. Service "to the public" is interpreted by EPA to include factories and private housing developments. (See generally, House Report, pp. 16–17.)

The definition of "public water system" proposed in the Interim Primary Drinking Water Regulations sought to explain the meaning of the statutory reference to "regular" service. It was proposed to interpret this term as including service for as much as three months during the year. Because the proposed definition would have excluded many large campgrounds, lodges, and other public accommodations which serve large numbers of tourists but which are open for slightly less than three months each year, the definition in the final version covers systems serving an average of at least twenty-five individuals at least 60 days out of the year. The use of a minimum number of days rather than months also makes clear that a system may qualify as a public water system even if it is not open every day during a given month.

Once "public water system" has been defined, it is necessary to define the two major types of public water systems—those serving residents and those serving transients or intermittent users. The possible health effects of a contaminant in drinking water in many cases are quite different for a person drinking the water for a long period of time than for a person drinking the water only briefly or intermittently. Different regulatory considerations may in some cases apply to systems which serve residents as opposed to systems which serve transients or intermittent users. Accordingly, § 141.2(e) makes clear that all "public water systems" fall within either the category of "community water systems" or the category of "non-community water systems." To make clear which regulatory requirements apply to which type of system, the category covered is specifically indicated throughout the regulations.

The proposed regulations defined a "community water system" as "a public water system which serves a population of which 70 percent or greater are residents." Reliance in the proposed definition on the percentage of water system users who are residents would result in treating some fairly large resort communities with many year-round residents as non-community systems. Therefore, the definition of "community water system" has been changed to cover any system which serves at least 15 service connections used by year-round residents or serves at least 25 year-round residents.

SMALL COMMUNITY WATER SYSTEMS

Many community water systems in the country are quite small. Since it is the intention of the Act to provide basically the same level of health protection to residents of small communities as to residents of large cities, and since a number of advanced water treatment techniques are made feasible only by economies of scale, the cost of compliance with the requirements of the Act may pose a serious problem for many small communities. The regulations seek to recognize the financial problems of small communities by requiring more realistic monitoring for systems serving fewer than 1,000 persons. Variances and exemptions authorized by the Act can also assist in dealing with economic problems of small community systems in appropriate cases, at least temporarily. EPA will provide technical assistance on effective treatment techniques which can be used by small systems.

These methods of dealing with the financial problems of some small community systems may not be sufficient in specific instances to make compliance with all applicable regulatory requirements feasible. EPA is commencing a study of potential problems faced by small community systems in meeting applicable requirements under the Act and these regulations, and, if necessary, will make additional adjustments in the Interim Primary Drinking Water Regulations prior to their effective date.

NON-COMMUNITY SYSTEMS

"Non-community systems" are basically those systems which serve transients. They include hotels, motels, restaurants, campgrounds, service stations, and other public accommodations which have their own water system and which have at least 15 service connections or serve water to a daily average of at least 25 persons. Some schools, factories and churches are also included in this category. It is conservatively estimated that there are over 200,000 non-community water systems in the country. However, it should be recognized that while their number is large, they normally are not the principal source of water for the people they serve.

The regulations as proposed would have applied all maximum contaminant levels to non-community systems as well as to community systems. This approach failed to take into account the fact that the proposed maximum contaminant levels for organic chemicals and most inorganic chemicals were based on the potential health effects of long-term exposure. Those levels are not necessary to protect transients or intermittent users. Therefore, the final regulations provide that maximum contaminant levels for organic chemicals, and for inorganic chemicals other than nitrates, are not applicable to non-community systems. An exception was made for nitrates because they can have an adverse health effect on susceptible infants in a short period of time.

Even without monitoring for organic chemicals or most inorganic chemicals, in the initial stages of implementation of the drinking water regulations, monitoring results from tens of thousands of non-community systems could overwhelm laboratory capabilities and other resources. This could delay effective implementation of the regulations with respect to the community systems which provide the water which Americans drink every day. To avoid this result, non-community systems will be given two years after the effective date of the regulations to commence monitoring. In the meantime, non-community systems which already monitor their water are encouraged to continue to do so, and the States are encouraged to take appropriate measures to test or require monitoring for non-community systems that serve large numbers of people.

Of course, non-community systems which pose a threat to health should be dealt with as quickly as possible. The maximum contaminant levels applicable to non-community water systems therefore will take effect 18 months after promulgation, at the same time as levels applicable to community systems. Inspection and enforcement authority will apply to non-community systems at the same time as to community systems.

SANITARY SURVEYS

EPA encourages the States to conduct sanitary surveys on a systematic basis.

These on-site inspections of water systems are more effective in assuring safe water to the public than individual tests taken in the absence of sanitary surveys. The regulations provide that monitoring frequencies for coliform bacteria can be changed by the entity with primary enforcement responsibility for an individual non-community system, and in certain circumstances for an individual community system, based on the results of a sanitary survey.

MAXIMUM CONTAMINANT LEVELS

Numerous comments were received by EPA on the substances selected for the establishment of maximum contaminant levels and on the levels chosen. Congress anticipated that the initial Interim Primary Drinking Water Regulations would be based on the Public Health Service Standards of 1962, and this Congressional intent has been followed. Comments received on the various levels did not contain new data sufficient to require the establishment of levels different from those contained in the Public Health Service Standards.

WATER CONSUMPTION

The maximum contaminant levels are based, directly or indirectly, on an assumed consumption of two liters of water per day. The same assumption was used in the 1962 Standards. This assumption has been challenged because of instances where much higher water consumption rates occur. EPA's justification for using the two-liter figure is that it already represents an above average water or water-based fluid intake. Moreover, while the factor of safety may be somewhat reduced when greater quantities of water are ingested, the maximum contaminant levels based on the two-liter figure provide substantial protection to virtually all consumers. If, as has been suggested, a water consumption rate of eight liters per day is used as the basis for maximum contaminant level, all of the proposed MCL's would have to be divided by four, greatly increasing the monitoring difficulties, and in some cases challenging the sensitivity of accepted analytical procedures. It could be expected, in such a case, that the maximum contaminant levels would be exceeded to a significant degree, and that specialized treatment techniques would be required to order that the contaminant levels would be reduced. The economic impact of a move in this direction would be enormous. It is not technically or economically feasible to base maximum contaminant levels on unusually high consumption rates.

SAFETY FACTORS

A question was raised about the fact that different safety factors are contained in various maximum contaminant levels. The levels are not intended to have a uniform safety factor, at least partly because the knowledge of and the nature of the health risks of the various contaminants vary widely. The levels set are the result of experience, evaluation of the available data, and professional judgment. They have withstood the test of time and of professional review. They are being subjected to further review by the National Academy of Sciences in connection with development of data for the Revised Primary Drinking Water Regulations.

MCL'S BASED ON TEMPERATURE

A question was also raised as to whether ranges of maximum contaminant levels should be established on the basis of the climate in the area served by the public water system, as was done with fluoride. EPA believes that the use of a temperature scale for fluoride is more appropriate than for other chemicals because of the studies available on the fluoride-temperature relationship and because there is a small margin with fluoride between beneficial levels and levels that cause adverse health effects.

MCL'S DELETED

Three proposed maximum contaminant levels have been eliminated in the final regulations because they are not justified by the available data. One of these is carbon chloroform extract (CCE), which is discussed separately below. The others are the proposed levels for the standard bacterial plate count and cyanide. In the case of the plate count, it is believed that the coliform limits contained in the regulations, combined with the turbidity maximum contaminant level, adequately deal with bacterial contamination. However, EPA continues to believe that the standard plate count is a valid indicator of bacteriological quality of drinking water, and recommends that it be used in appropriate cases in conjunction with the coliform tests as an operational tool.

The proposed maximum contaminant level for cyanide was eliminated because the possibility of cyanide contamination can be effectively addressed only by the use of emergency action, such as under Section 1431 of the Act. EPA's 1969 Community Water Supply Study did not reveal a single instance in which cyanide was present in a water system at a level greater than one-thousandth of the level at which cyanide is toxic to humans.

Available data indicate that cyanide will be present in water systems at toxic levels only in the event of an accident, such as a spill from a barge collision. Maximum contaminant levels are not the appropriate vehicle for dealing with such rare, accidental contamination.

Heptachor, heptachlor epoxide and chlordane have also been removed from the list of maximum contaminant levels at least temporarily in view of the pending cancellation and suspension proceedings under the Federal Insecticide, Fungicide and Rodenticide Act involving those pesticides. When the results of these proceedings are available, EPA will again consider whether maximum contaminant levels should be established for those three pesticides.

SODIUM AND SULFATES

A number of comments were received on the potential health effects of sodium and sulfates. The National Drinking Water Advisory Council has recommended that consideration be given to the monitoring of these constituents, but has not recommended the adoption of maximum contaminant levels because available data do not support the adoption of any specific levels. EPA has requested the National Academy of Sciences to include sodium and sulfates among the contaminants to be studied by NAS, and to include information on the health effects of sodium and sulfates in the report to be made by NAS in December 1976.

Since a number of persons suffer from diseases which are influenced by dietary sodium intake and since there are others who wish to restrict their sodium intake, it is desirable that the sodium content of drinking water be known. Those affected can, by knowing the sodium concentration in their drinking water, make adjustments to their diets or, in extreme cases, seek alternative sources of water to be used for drinking and food preparation. It is recommended that the States institute programs for regular monitoring of the sodium content of drinking water served to the public, and for informing physicians and consumers of the sodium concentration in drinking water.

A relatively high concentration of sulfate in drinking water has little or no known laxative effect on regular users of the water, but transcients using such water sometimes experience a laxative effect. It is recommended that the States institute monitoring programs for sulfates, and that transients be notified if the sulfate content of the water is high. Such notification should include an assessment of the possible physiological effects of consumption of the water.

PCB'S AND ASBESTOS

An interagency comment expressed concern for asbestos and PCB's in the environment and noted the need for at least a monitoring requirement, if not for MCL's, for these contaminants. EPA is also concerned, but for the moment lacks sufficient evidence regarding analytical methods, health effects, or occurrence in the environment to establish MCL's. The Agency is conducting research and cooperating in research projects to develop criteria for establishing needed limits as quickly as possible. A monitoring study on a number of organic chemical contaminants, including PCB's, for which MCL's are not being established at this time, will be contained in an organic chemical monitoring regulation that is being promulgated with these regulations. Regarding asbestos, HEW and EPA are sponsoring a number of studies this year at an approximate cost of $16 million to establish health effects analytical methods and occurrence.

POINT OF MEASUREMENT

Other comments on maximum contaminant levels focused on the proposed requirement that such levels be tested at the consumer's tap. Concern was expressed over the inability of the public water system to control potential sources of contaminants which are under the control of the consumer.

The promulgated definition of "maximum contaminant level," § 141.2(d), retains the requirement that the maximum contaminant level be measured at the tap except in the case of turbidity, which should be measured at the point of entry to the distribution system. However, the definition has been expanded to make clear that contaminants added to the water by circumstances under the control of the consumer are not the responsibility of the supplier of water, unless the contaminants result from corrosion of piping and plumbing resulting from the quality of the water supplied. It should be noted, however, that this requirement should not be interpreted as to discourage local, aggressive cross connection control measures.

COLIFORM BACTERIA MCL'S

The promulgated MCL's for coliform bacteria are basically the 1962 Public Health Service Standards, with minor refinements and clarifications. However, further changes may be desirable. For example, the MCL's for the membrane filter analytical method do not resolve the question of how many coliform bacteria are assumed to be present in a single highly contaminated sample. Some laboratories assume an upper limit of 50, while others seek to continue to count individual bacteria to a level of 100 or even higher in a single sample. The upper limit assumed will affect the monthly average which is calculated to determine compliance with the MCL's.

Another question relating to the coliform bacteria MCL's is the matter of possible spurious positive samples. As the regulations are written, all routine samples taken to determine compliance with the MCL's must be counted, regardless of the results of analysis of any check samples that may be taken. The reason for this is that bacterial contamination is often intermittent or transient, and as a result negative check samples taken a day or more after a positive sample cannot demonstrate that the positive result was in error. It may be possible, however, to prescribe a means of dealing with spurious positive results without compromising the integrity of the MCL's.

A third question concerning the MCL's for coliform bacteria is the relationship of monthly averages of coliform bacteria levels to monthly percentages of positive samples. For example, the monthly average MCL for the membrane filter method is violated if the monthly average exceeds one coliform bacterium per sample. However, for purposes of determining whether the monthly-percentage-of-positive-samples MCL is violated, a sample is counted as positive only if it contains more than four coliform bacteria. Thus, it is possible, particularly when a relatively small number of samples is taken, for a system to fail the monthly average MCL even when no single sample taken during the month is out of compliance with the limit.

These and other questions concerning the coliform bacteria MCL's will be reviewed further by EPA. If review indicates that changes in the MCL's are desirable, those changes will be made as soon as possible but within 6 months, in time to take effect at the same time as the initial Interim Primary Drinking Water Regulations.

ORGANIC CHEMICALS

The proposed maximum contaminant levels for organic pesticides, other than the three which are the subject of cancellation and suspension proceedings, have been retained. It is anticipated that additional organic pesticides will be added to the regulations if surveys of pesticides in drinking water being conducted by EPA indicate that this is needed.

The proposed regulations also contained a maximum contaminant level for organic chemicals obtained by the carbon chloroform extract (CCE) method. It was anticipated by Congress that organic chemicals would be dealt with primarily in the Revised Primary Drinking Water Regulations because of the paucity of accurate data on the health effects of various organic chemicals, the large number of such chemicals, uncertainities over appropriate treatment techniques, and the need for additional information on the incidence of specific organic chemicals in drinking water supplies. EPA thought that the CCE standard might provide an appropriate means of dealing with organic chemicals as a class pending action on the Revised Primary Regulations.

The CCE standard was originally developed as a test for undesirable tastes and odors in drinking water. As concern developed over the health effects of organic chemicals, the possibility of using CCE as a health standard rather than an esthetic standard was considered.

As pointed out by numerous comments, CCE has many failings as an indicator of health effects of organic chemicals. To begin with, the test obtains information on only a fraction of the total amount of organic chemicals in the water sampled. Furthermore, there is serious question as to the reliability of CCE in identifying those organic chemicals which are most suspected of adverse health effects. In addition, there are no existing data on which a specific level for CCE can be established on a rational basis. To establish a maximum contaminant level under these circumstances would almost certainly do more harm than good. It could give a false sense of security to persons served by systems which are within the established level and a false sense of alarm to persons served by systems which exceed the level. It also would divert resources from efforts to find more effective ways of dealing with the organic chemicals problem.

EPA believes that the intelligent approach to the organic chemicals question is to move ahead as rapidly as possible along two fronts. First, EPA is adopting simultaneously with these regulations a Subpart E of Part 141, containing requirements for organic chemical monitoring pursuant to Sections 1445 and 1450 of the Act.

The regulations require that designated public water systems collect samples of raw and treated water for submission to EPA for organics analysis. EPA will analyze the samples for a number of broad organic parameters, including carbon chloroform extract (CCE), volatile and non-volatile total organic carbon (VTOC and NVTOC), total organic chlorine (TOCl), ultraviolet absorbancy, and fluorescence. In addition, monitoring will be required for probably 21 specific organic compounds. Selection of the specific compounds has been based on the occurrence or likelihood of occurrence in treated water, toxicity data and availability of practical analytical methods. Laboratory analyses will be used to evaluate the extent and nature of organic chemical contamination of drinking water, to evaluate the validity of the general organic parameters as surrogates for measures of harmful organic chemicals, and to determine whether there is an adequate basis for establishing maximum contaminant levels for specific organics or groups of organics.

Second, EPA is embarking on an intensive research program to find answers to the following four questions:

1. What are the effects of commonly occurring organic compounds on human health?

2. What analytical procedures should be used to monitor finished drinking water to assure that any Primary Drinking Water Regulations dealing with organics are met?

3. Because some of these organic compounds are formed during water treatment, what changes in treatment practices are required to minimize the formation of these compounds in treated water?

4. What treatment technology must be applied to reduce contaminant levels to concentrations that may be specified in the Primary Drinking Water Regulations?

This research will involve health-effects and epidemiological studies, investigations of analytical methodology, and pilot plant and field studies of organic removal unit processes. Some phases of the research are to be completed by the end of this year, while much of the remainder are to be completed within the next calendar year.

As soon as sufficient information is derived from the monitoring program and related research, the Interim Primary Drinking Water Regulations will be amended so that the organic chemicals problem can be dealt with without delay. The monitoring process will be completed within 1 year.

During the interim period, while satisfactory MCL's for organic contamination in drinking water are being developed, EPA will act in specific cases where appropriate to deal with organic contamination. If the EPA monitoring program reveals serious specific cases of contamination, EPA will work with State and local authorities to identify the source and nature of the problem and to take remedial action. EPA will also aid the States in identifying additional community water supplies that require analysis.

PUBLIC NOTICE

The public notice requirements proposed in § 141.32 did not distinguish between community and non-community public water systems. They would have required that public notice of non-compliance with applicable regulations be made by newspaper, in water bills, and by other media for all public water systems. These requirements are inappropriate and ineffective in the case of most non-community water systems. Those systems principally serve transients who do not receive water bills from the system and who probably are not exposed significantly to the local media. A more effective approach would be to require notice that can inform the transient before he drinks the system's water, and thereby both warn the transient and provide an incentive to the supplier of water to remedy the violation. Accordingly, Section 141.32 as adopted provides that in the case of non-community systems, the entity with primary enforcement responsibility shall require that notice be given in a form and manner that will insure that the public using the public water system is adequately informed.

The proposed public notice requirements also failed to distinguish between different types of violations of the Interim Primary Drinking Water Regulations. Since the urgency and importance of a notice varies according to the nature of the violation involved, § 141.32 as promulgated seeks to match the type of notice required with the type of violation involved. Written notice accompanying a water bill or other direct notice by mail is required for all violations of the regulations, including violations of monitoring requirements, and for the grant of a variance or exemption. In addition, notice by newspaper and notification to radio and television stations is required whenever a maximum contaminant level is exceeded, or when the entity with primary enforcement responsibility requires such broader notice.

QUALITY CONTROL AND TESTING PROCEDURES

Section 1401(1) of the Act defines "primary drinking water regulation" to include "quality control and testing procedures." The promulgated regulations include testing requirements for each maximum contaminant level, including check samples and special samples in appropriate cases. The regulations also specify the procedures to be followed in analyzing samples for each of the maximum contaminant levels. These procedures will be updated from time to time as advances are made in analytical methods. For example, references to "Standard Methods for the Examination of Water and Wastewater" are to the current, 13th, edition, but these references will be changed to cite the 14th edition when it is available in the near future.

A key element of quality control for public water systems is accurate laboratory analysis. Section 141.28 of the regulations provides that analyses conducted for the purpose of determining compliance with maximum contaminant levels must be conducted by a laboratory approved by the entity with primary enforcement responsibility. EPA will develop as soon as possible, in cooperation with the States and other interested parties, criteria and procedures for laboratory certification. A State with primary enforcement responsibility will have a laboratory certified by EPA pursuant to the prescribed criteria and procedures, and in turn will certify laboratories within the State.

Record-keeping requirements and reports to the State also will assist in quality control efforts.

RECORD-KEEPING

Adequate record-keeping is necessary for the proper operation and administration of a public water system. It is also important for providing information to the public, providing appropriate data for inspection and enforcement activities and providing information on which future regulations can be based. Accordingly, a new § 141.33 has been added to the regulations to require that each public water system maintain records of sample analyses and of actions to correct violations of the Primary Drinking Water Regulations.

ECONOMIC AND COST ANALYSIS

A comprehensive economics study has been made of the Interim Primary Drinking Water Regulations. This study estimates the costs of the regulations, evaluates the potential economic impact, and considers possible material and labor shortages. The results of this analysis are summarized here.

Total investment costs to community water systems to achieve compliance with these regulations are estimated to be between $1,050 and $1,765 million. It is estimated that non-community systems will invest an additional $24 million. The range of the estimate is due to uncertainty as to the design flow that will be used in installing treatment facilities. Systems not in compliance will have to consider sizing their new components to reflect average daily flow conditions, or maximum daily flow conditions in cases where system storage is not adequate.

This investment will be spread over several years. Investor-owned systems will bear about one-fourth of these costs, and publicly-owned systems the remainder. It is not anticipated that systems will have difficulty financing these capital requirements.

In annual terms, national costs are expected to be within the following ranges:

	In millions
Capital costs	$146–247
Operations and maintenance	263–263
Monitoring (routine only)	17– 35
Total	$426–545

Although these aggregate figures are large, most water consumers will not be significantly affected. For those users in systems serving 10,000 persons or more, the average annual treatment cost per capita may increase from less than $1.00 for systems requiring disinfection and lead control, to between $15 to $35 for control of turbidity and heavy metal removal. For systems serving less than 100 persons, the average annual per capita costs of disinfection, lead control and fluoride/arsenic removal are estimated to be between $2.10 and $11.80. However, if turbidity control or heavy metal removal were required in a system of this size then costs are expected to range from $52 to $237 per year per capita. EPA is aware of the serious potential economic impact on users in these small systems. However, the legislative history specifies that the regulations should be based on costs that can be reasonably afforded by large metropolitan or regional systems. Further economic evaluation of these systems is being conducted, and realistic options for these small systems are being reviewed. Options that will be under consideration include less costly treatment technologies; formation of regional systems; and use of alternative water sources. Industrial and commercial users, whether providing their own water or using public systems, are not expected to be significantly affected by these regulations.

Possible constraints to the implementation of the interim primary regulations were examined. Although there will be an increase in demand for chemicals, manpower, laboratories, and construction of treatment facilities, it is not anticipated that any of these factors will be a serious obstacle to implementation of these regulations over a reasonable time frame.

For the reasons given above, Chapter 40 of the Code of Federal Regulations is hereby amended by the addition of the following new Part 141. These regulations will take effect 18 months after promulgation.

(It is hereby certified that the economic and inflationary impacts of these regulations have been carefully evaluated in accordance with Executive Order 11821)

Dated: December 10, 1975.

RUSSELL E. TRAIN,
Administrator.

Subpart A—General

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Subpart D—Reporting, Public Notification, and Record-keeping

141.31 Reporting requirements.
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AUTHORITY: Secs. 1412, 1414, 1445, and 1450 of the Public Health Service Act, 88 Stat. 1660 (42 U.S.C. 300g-1, 300g-3, 300j-4, and 300j-9).

Subpart A—General

§ 141.1 Applicability.

This part establishes primary drinking water regulations pursuant to section 1412 of the Public Health Service Act, as amended by the Safe Drinking Water Act (Pub. L. 93-523); and related regulations applicable to public water systems.

§ 141.2 Definitions.

As used in this part, the term:

(a) "Act" means the Public Health Service Act, as amended by the Safe Drinking Water Act, Pub. L. 93-523.

(b) "Contaminant" means any physical, chemical, biological, or radiological substance or matter in water.

(c) "Maximum contaminant level" means the maximum permissible level of a contaminant in water which is delivered to the free flowing outlet of the ultimate user of a public water system, except in the case of turbidity where the maximum permissible level is measured at the point of entry to the distribution system. Contaminants added to the water under circumstances controlled by the user, except those resulting from corrosion of piping and plumbing caused by water quality, are excluded from this definition.

(d) "Person" means an individual, corporation, company, association, partnership, State, municipality, or Federal agency.

(e) "Public water system" means a system for the provision to the public of piped water for human consumption, if such system has at least fifteen service connections or regularly serves an average of at least twenty-five individuals daily at least 60 days out of the year. Such term includes (1) any collection, treatment, storage, and distribution facilities under control of the operator of such system and used primarily in connection with such system, and (2) any collection or pretreatment storage facilities not under such control which are used primarily in connection with such system. A public water system is either a "community water system" or a "non-community water system."

(i) "Community water system" means a public water system which serves at least 15 service connections used by year-round residents or regularly serves at least 25 year-round residents.

(ii) "Non-community water system" means a public water system that is not a community water system.

(f) "Sanitary survey" means an on-site review of the water source, facilities, equipment, operation and maintenance of a public water system for the purpose of evaluating the adequacy of such source, facilities, equipment, operation and maintenance for producing and distributing safe drinking water.

(g) "Standard sample" means the aliquot of finished drinking water that is examined for the presence of coliform bacteria.

(h) "State" means the agency of the State government which has jurisdiction over public water systems. During any period when a State does not have primary enforcement responsibility pursuant to Section 1413 of the Act, the term "State" means the Regional Administrator, U.S. Environmental Protection Agency.

(i) "Supplier of water" means any person who owns or operates a public water system.

§ 141.3 Coverage.

This part shall apply to each public water system, unless the public water system meets all of the following conditions:

(a) Consists only of distribution and storage facilities (and does not have any collection and treatment facilities);

(b) Obtains all of its water from, but is not owned or operated by, a public water system to which such regulations apply;

(c) Does not sell water to any person; and

(d) Is not a carrier which conveys passengers in interstate commerce.

§ 141.4 Variances and exemptions.

Variances or exemptions from certain provisions of these regulations may be granted pursuant to Sections 1415 and 1416 of the Act by the entity with primary enforcement responsibility. Provisions under Part 142, *National Interim Primary Drinking Water Regulations Implementation*—subpart E (Variances) and subpart F (Exemptions)—apply where EPA has primary enforcement responsibility.

§ 141.5 Siting requirements.

Before a person may enter into a financial commitment for or initiate construction of a new public water system or increase the capacity of an existing public water system, he shall notify the State and, to the extent practicable, avoid locating part or all of the new or expanded facility at a site which:

(a) Is subject to a significant risk from earthquakes, floods, fires or other disasters which could cause a breakdown of the public water system or a portion thereof; or

(b) Except for intake structures, is within the floodplain of a 100-year flood or is lower than any recorded high tide where appropriate records exist.

The U.S. Environmental Protection Agency will not seek to override land use decisions affecting public water systems siting which are made at the State or local government levels.

§ 141.6 Effective date.

The regulations set forth in this part shall take effect 18 months after the date of promulgation.

Subpart B—Maximum Contaminant Levels

§ 141.11 Maximum contaminant levels for inorganic chemicals.

(a) The maximum contaminant level for nitrate is applicable to both community water systems and non-community water systems. The levels for the other inorganic chemicals apply only to community water systems. Compliance with maximum contaminant levels for inorganic chemicals is calculated pursuant to § 141.23.

(b) The following are the maximum contaminant levels for inorganic chemicals other than fluoride:

Contaminant	*Level, milligrams per liter*
Arsenic	0.05
Barium	1.
Cadmium	0.010
Chromium	0.05
Lead	0.05
Mercury	0.002
Nitrate (as N)	10.
Selenium	0.01
Silver	0.05

(c) When the annual average of the maximum daily air temperatures for the location in which the community water system is situated is the following, the maximum contaminant levels for fluoride are:

Temperature Degrees Fahrenheit	Degrees Celsius —	Level, milligrams per liter
53.7 and below	12.0 and below	2.4
53.8 to 58.3	12.1 to 14.6	2.2
58.4 to 63.8	14.7 to 17.6	2.0
63.9 to 70.6	17.7 to 21.4	1.8
70.7 to 79.2	21.5 to 26.2	1.6
79.3 to 90.5	26.3 to 32.5	1.4

§ 141.12 Maximum contaminant levels for organic chemicals.

The following are the maximum contaminant levels for organic chemicals. They apply only to community water systems. Compliance with maximum contaminant levels for organic chemicals is calculated pursuant to § 141.24.

	Level, milligrams per liter
(a) Chlorinated hydrocarbons:	
Endrin (1,2,3,4,10, 10-hexachloro-6,7-epoxy-1,4, 4a,5,6,7,8,8a-octahydro-1,4-endo, endo-5,8 - dimethano naphthalene).	0.0002
Lindane (1,2,3,4,5,6-hexachlorocyclohexane, gamma isomer).	0.004
Methoxychlor (1,1,1-Trichloro-2, 2 - bis [p-methoxyphenyl] ethane).	0.1
Toxaphene ($C_{10}H_{10}Cl_8$-Technical chlorinated camphene, 67–69 percent chlorine).	0.005

(b) Chlorophenoxys:

2,4 - D, (2,4-Dichlorophenoxyacetic acid).	0.1
2,4,5-TP Silvex (2,4,5-Trichlorophenoxypropionic acid).	0.01

§ 141.13 Maximum contaminant levels for turbidity.

The maximum contaminant levels for turbidity are applicable to both community water systems and non-community water systems using surface water sources in whole or in part. The maximum contaminant levels for turbidity in drinking water, measured at a representative entry point(s) to the distribution system, are:

(a) One turbidity unit (TU), as determined by a monthly average pursuant to § 141.22, except that five or fewer turbidity units may be allowed if the supplier of water can demonstrate to the State that the higher turbidity does not do any of the following:

(1) Interfere with disinfection;

(2) Prevent maintenance of an effective disinfectant agent throughout the distribution system; or

(3) Interfere with microbiological determinations.

(b) Five turbidity units based on an average for two consecutive days pursuant to § 141.22.

§ 141.14 Maximum microbiological contaminant levels.

The maximum contaminant levels for coliform bacteria, applicable to community water systems and non-community water systems, are as follows:

(a) When the membrane filter technique pursuant to § 141.21(a) is used, the number of coliform bacteria shall not exceed any of the following:

(1) One per 100 milliliters as the arithmetic mean of all samples examined per month pursuant to § 141.21 (b) or (c);

(2) Four per 100 milliliters in more than one sample when less than 20 are examined per month; or

(3) Four per 100 milliliters in more than five percent of the samples when 20 or more are examined per month.

(b)(1) When the fermentation tube method and 10 milliliter standard portions pursuant to § 141.21(a) are used, coliform bacteria shall not be present in any of the following:

(i) more than 10 percent of the portions in any month pursuant to § 141.21 (b) or (c);

(ii) three or more portions in more than one sample when less than 20 samples are examined per month; or

(iii) three or more portions in more than five percent of the samples when 20 or more samples are examined per month.

(2) When the fermentation tube method and 100 milliliter standard portions pursuant to § 141.21(a) are used, coliform bacteria shall not be present in any of the following:

(i) more than 60 percent of the portions in any month pursuant to § 141.21 (b) or (c);

(ii) five portions in more than one sample when less than five samples are examined per month; or

(iii) five portions in more than 20 percent of the samples when five or more samples are examined per month.

(c) For community or non-community systems that are required to sample at a rate of less than 4 per month, compliance with paragraphs (a), (b)(1), or (b)(2) of this section shall be based upon sampling during a 3 month period, except that, at the discretion of the State, compliance may be based upon sampling during a one-month period.

Subpart C—Monitoring and Analytical Requirements

§ 141.21 Microbiological contaminant sampling and analytical requirements.

(a) Suppliers of water for community water systems and non-community water systems shall analyze for coliform bacteria for the purpose of determining compliance with § 141.14. Analyses shall be conducted in accordance with the analytical recommendations set forth in "Standard Methods for the Examination of Water and Wastewater," American Public Health Association, 13th Edition, pp. 662–688, except that a standard sample size shall be employed. The standard sample used in the membrane filter procedure shall be 100 milliliters. The standard sample used in the 5 tube most probable number (MPN) procedure (fermentation tube method) shall be 5 times the standard portion. The standard portion is either 10 milliliters or 100 milliliters as described in § 141.14 (b) and (c). The samples shall be taken at points which are representative of the conditions within the distribution system.

(b) The supplier of water for a community water system shall take coliform density samples at regular time intervals, and in number proportionate to the population served by the system. In no event shall the frequency be less than as set forth below:

Population served:	*Minimum number of samples per month*
25 to 1,000	1
1,001 to 2,500	2
2,501 to 3,300	3
3,301 to 4,100	4
4,101 to 4,900	5
4,901 to 5,800	6
5,801 to 6,700	7
6,701 to 7,600	8
7,601 to 8,500	9
8,501 to 9,400	10
9,401 to 10,300	11
10,301 to 11,100	12
11,101 to 12,000	13
12,001 to 12,900	14
12,901 to 13,700	15
13,701 to 14,600	16
14,601 to 15,500	17
15,501 to 16,300	18
16,301 to 17,200	19
17,201 to 18,100	20
18,101 to 18,900	21
18,901 to 19 800	22
19,801 to 20,700	23
20,701 to 21,500	24
21,501 to 22,300	25
22,301 to 23,200	26
23.201 to 24,000	27
24,001 to 24,900	28
24,901 to 25,000	29
25,001 to 28,000	30
28,001 to 33,000	35
33,001 to 37,000	40
37,001 to 41,000	45
41,001 to 46,000	50
46.001 to 50,000	55
50,001 to 54,000	60
54,001 to 59,000	65
59,001 to 64,000	70
64,001 to 70,000	75
70,001 to 76,000	80
76,001 to 83,000	85
83,001 to 90,000	90
90,001 to 96,000	95
96,001 to 111,000	100
111,001 to 130,000	110
130,001 to 160,000	120
160,001 to 190,000	130
190,001 to 220,000	140
220,001 to 250,000	150
250,001 to 290,000	160
290,001 to 320,000	170
320,001 to 360,000	180
360,001 to 410,000	190
410,001 to 450,000	200
450,001 to 500,000	210
500,001 to 550,000	220
550,001 to 600,000	230
600.001 to 660,000	240
660,001 to 720,000	250
720,001 to 780,000	260
780,001 to 840,000	270
840,001 to 910,000	280
910,001 to 970,000	290
970,001 to 1,050,000	300
1,050,001 to 1,140,000	310
1,140,001 to 1,230,000	320
1,230,001 to 1,320,000	330
1,320,001 to 1,420,000	340
1,420,001 to 1,520,000	350
1,520,001 to 1,630,000	360
1,630,001 to 1,730,000	370
1,730,001 to 1,850,000	380
1,850,001 to 1,970,000	390
1,970,001 to 2,060,000	400
2,060,001 to 2,270,000	410
2,270,001 to 2,510,000	420
2,510,001 to 2,750,000	430
2,750,001 to 3,020,000	440
3,020,001 to 3,320,000	450
3,320,001 to 3,620,000	460
3,620,001 to 3,960,000	470
3,960,001 to 4,310,000	480
4,310,001 to 4,690 000	490
4,690,001 or more	500

Based on a history of no coliform bacterial contamination and on a sanitary survey by the State showing the water system to be supplied solely by a protected ground water source and free of sanitary defects, a community water system serving 25 to 1,000 persons, with written permission from the State, may reduce this sampling frequency except that in no case shall it be reduced to less than one per quarter.

(c) The supplier of water for a non-community water system shall sample for coliform bacteria in each calendar quarter during which the system provides water to the public. Such sampling shall begin within two years after the effective date of this part. If the State, on the basis of a sanitary survey, determines that some other frequency is more appropriate, that frequency shall be the frequency required under these regulations. Such frequency shall be confirmed or changed on the basis of subsequent surveys.

(d)(1) When the coliform bacteria in a single sample exceed four per 100 milliliters (§ 141.14(a)), at least two consecutive daily check samples shall be collected and examined from the same sampling point. Additional check samples shall be collected daily, or at a frequency established by the State, until the results obtained from at least two consecutive check samples show less than one coliform bacterium per 100 milliliters.

(2) When coliform bacteria occur in three or more 10 ml portions of a single sample (§ 141.14(b)(1)), at least two consecutive daily check samples shall be collected and examined from the same sampling point. Additional check samples shall be collected daily, or at a frequency established by the State, until the results obtained from at least two consecutive check samples show no positive tubes.

(3) When coliform bacteria occur in all five of the 100 ml portions of a single sample (§ 141.14(b)(2)), at least two daily check samples shall be collected and examined from the same sampling point. Additional check samples shall be collected daily, or at a frequency established by the State, until the results obtained from at least two consecutive check samples show no positive tubes.

(4) The location at which the check samples were taken pursuant to paragraphs (d) (1), (2), or (3) of this section shall not be eliminated from future sampling without approval of the State. The results from all coliform bacterial analyses performed pursuant to this subpart, except those obtained from check samples and special purpose samples, shall be used to determine compliance with the maximum contaminant level for coliform bacteria as established in § 141.14. Check samples shall not be included in calculating the total number of samples taken each month to determine compliance with § 141.21 (b) or (c).

(e) When the presence of coliform bacteria in water taken from a particular sampling point has been confirmed by any check samples examined as directed in paragraphs (d) (1), (2), or (3) of this section, the supplier of water shall report to the State within 48 hours.

(f) When a maximum contaminant level set forth in paragraphs (a), (b) or (c) of § 141.14 is exceeded, the supplier of water shall report to the State and notify the public as prescribed in § 141.31 and § 141.32.

(g) Special purpose samples, such as those taken to determine whether disinfection practices following pipe placement, replacement, or repair have been sufficient, shall not be used to determine compliance with § 141.14 or § 141.21 (b) or (c).

(h) A supplier of water of a community water system or a non-community water system may, with the approval of the State and based upon a sanitary survey, substitute the use of chlorine residual monitoring for not more than 75 percent of the samples required to be taken by paragraph (b) of this section, *Provided*, That the supplier of water takes chlorine residual samples at points which are representative of the conditions within the distribution system at the frequency of at least four for each substituted microbiological sample. There shall be at least daily determinations of chlorine residual. When the supplier of water exercises the option provided in this paragraph (h) of this section, he shall maintain no less than 0.2 mg/l free chlorine throughout the public water distribution system. When a particular sampling point has been shown to have a free chlorine residual less than 0.2 mg/l, the water at that location shall be retested as soon as practicable and in any event within one hour. If the original analysis is confirmed, this fact shall be reported to the State within 48 hours. Also, if the analysis is confirmed, a sample for coliform bacterial analysis must be collected from that sampling point as soon as practicable and preferably within one hour, and the results of such analysis reported to the State within 48 hours after the results are known to the supplier of water. Analyses for residual chlorine shall be made in accordance with "Standard Methods for the Examination of Water and Wastewater," 13th Ed., pp. 129–132. Compliance with the maximum contaminant levels for coliform bacteria shall be determined on the monthly mean or quarterly mean basis specified in § 141.14, including those samples taken as a result of failure to maintain the required chlorine residual level. The State may withdraw its approval of the use of chlorine residual substitution at any time.

§ 141.22 Turbidity sampling and analytical requirements.

(a) Samples shall be taken by suppliers of water for both community water systems and non-community water systems at a representative entry point(s) to the water distribution system at least once per day, for the purpose of making turbidity measurements to determine compliance with § 141.13. The measurement shall be made by the Nephelometric Method in accordance with the recommendations set forth in "Standard Methods for the Examination of Water and Wastewater," American Public Health Association, 13th Edition, pp. 350–353, or "Methods for Chemical Analysis of Water and Wastes," pp. 295–298, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(b) If the result of a turbidity analysis indicates that the maximum allowable limit has been exceeded, the sampling and measurement shall be confirmed by resampling as soon as practicable and preferably within one hour. If the repeat sample confirms that the maximum allowable limit has been exceeded, the supplier of water shall report to the State within 48 hours. The repeat sample shall be the sample used for the purpose of calculating the monthly average. If the monthly average of the daily samples exceeds the maximum allowable limit, or if the average of two samples taken on consecutive days exceeds 5 TU, the supplier of water shall report to the State and notify the public as directed in § 141.31 and § 141.32.

(c) Sampling for non-community water systems shall begin within two years after the effective date of this part.

(d) The requirements of this § 141.22 shall apply only to public water systems which use water obtained in whole or in part from surface sources.

§ 141.23 Inorganic chemical sampling and analytical requirements.

(a) Analyses for the purpose of determining compliance with § 141.11 are required as follows:

(1) Analyses for all community water systems utilizing surface water sources shall be completed within one year following the effective date of this part. These analyses shall be repeated at yearly intervals.

(2) Analyses for all community water systems utilizing only ground water sources shall be completed within two years following the effective date of this part. These analyses shall be repeated at three-year intervals.

(3) For non-community water systems, whether supplied by surface or ground water sources, analyses for nitrate shall be completed within two years following the effective date of this part. These analyses shall be repeated at intervals determined by the State.

(b) If the result of an analysis made pursuant to paragraph (a) indicates that the level of any contaminant listed in § 141.11 exceeds the maximum contaminant level, the supplier of water shall report to the State within 7 days and initiate three additional analyses at the same sampling point within one month.

(c) When the average of four analyses made pursuant to paragraph (b) of this section, rounded to the same number of significant figures as the maximum contaminant level for the substance in question, exceeds the maximum contaminant level, the supplier of water shall notify the State pursuant to § 141.31 and give notice to the public pursuant to § 141.32. Monitoring after public notification shall be at a frequency designated by the State and shall continue until the maximum contaminant level has not been exceeded in two successive samples or until a monitoring schedule as a condition to a variance, exemption or enforcement action shall become effective.

(d) The provisions of paragraphs (b) and (c) of this section notwithstanding, compliance with the maximum contaminant level for nitrate shall be determined on the basis of the mean of two analyses. When a level exceeding the maximum contaminant level for nitrate is found, a second analysis shall be initiated within 24 hours, and if the mean of the two analyses exceeds the maximum contaminant level, the supplier of water shall report his findings to the State pursuant to § 141.31 and shall notify the public pursuant to § 141.32.

(e) For the initial analyses required by paragraph (a)(1), (2) or (3) of this section, data for surface waters acquired within one year prior to the effective date and data for ground waters acquired within 3 years prior to the effective date of this part may be substituted at the discretion of the State.

(f) Analyses conducted to determine compliance with § 141.11 shall be made in accordance with the following methods:

(1) Arsenic—Atomic Absorption Method, "Methods for Chemical Analysis of Water and Wastes," pp. 95–96, Environ-

mental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(2) Barium—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210-215, or "Methods for Chemical Analysis of Water and Wastes," pp. 97-98, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(3) Cadmium—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210-215, or "Methods for Chemical Analysis of Water and Wastes," pp. 101-103, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(4) Chromium—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210-215, or "Methods for Chemical Analysis of Water and Wastes," pp. 105-106, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(5) Lead—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210-215, or "Methods for Chemical Analysis of Water and Wastes," pp. 112-113, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(6) Mercury—Flameless Atomic Absorption Method, "Methods for Chemical Analysis of Water and Wastes," pp. 118-126, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(7) Nitrate—Brucine Colorimetric Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 461-464, or Cadmium Reduction Method, "Methods for Chemical Analysis of Water and Wastes," pp. 201-206, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(8) Selenium—Atomic Absorption Method, "Methods for Chemical Analysis of Water and Wastes," p. 145, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(9) Silver—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater", 13th Edition, pp. 210-215, or "Methods for Chemical Analysis of Water and Wastes", p. 146, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(10) Fluoride—Electrode Method, "Standard Methods for the Examination of Water and Wastewater", 13th Edition, pp. 172-174, or "Methods for Chemical Analysis of Water and Wastes," pp. 65-67, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974, or Colorimetric Method with Preliminary Distillation, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 171-172 and 174-176, or "Methods for Chemical Analysis of Water and Wastes," pp. 59-60, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

§ 141.24 Organic chemical sampling and analytical requirements.

(a) An analysis of substances for the purpose of determining compliance with § 141.12 shall be made as follows:

(1) For all community water systems utilizing surface water sources, analyses shall be completed within one year following the effective date of this part. Samples analyzed shall be collected during the period of the year designated by the State as the period when contamination by pesticides is most likely to occur. These analyses shall be repeated at intervals specified by the State but in no event less frequently than at three year intervals.

(2) For community water systems utilizing only ground water sources, analyses shall be completed by those systems specified by the State.

(b) If the result of an analysis made pursuant to paragraph (a) of this section indicates that the level of any contaminant listed in § 141.12 exceeds the maximum contaminant level, the supplier of water shall report to the State within 7 days and initiate three additional analyses within one month.

(c) When the average of four analyses made pursuant to paragraph (b) of this section, rounded to the same number of significant figures as the maximum contaminant level for the substance in question, exceeds the maximum contaminant level, the supplier of water shall report to the State pursuant to § 141.31 and give notice to the public pursuant to § 141.32. Monitoring after public notification shall be at a frequency designated by the State and shall continue until the maximum contaminant level has not been exceeded in two successive samples or until a monitoring schedule as a condition to a variance, exemption or enforcement action shall become effective.

(d) For the initial analysis required by paragraph (a) (1) and (2) of this section, data for surface water acquired within one year prior to the effective date of this part and data for ground water acquired within three years prior to the effective date of this part may be substituted at the discretion of the State.

(e) Analyses made to determine compliance with § 141.12(a) shall be made in accordance with "Method for Organochlorine Pesticides in Industrial Effluents," MDQARL, Environmental Protection Agency, Cincinnati, Ohio, November 28, 1973.

(f) Analyses made to determine compliance with § 141.12(b) shall be conducted in accordance with "Methods for Chlorinated Phenoxy Acid Herbicides in Industrial Effluents," MDQARL, Environmental Protection Agency, Cincinnati, Ohio, November 28, 1973.

§ 141.27 Alternative analytical techniques.

With the written permission of the State, concurred in by the Administrator of the U.S. Environmental Protection Agency, an alternative analytical technique may be employed. An alternative technique shall be aceeptable only if it is substantially equivalent to the prescribed test in both precision and accuracy as it relates to the determination of compliance with any maximum contaminant level. The use of the alternative analytical technique shall not decrease the frequency of monitoring required by this part.

§ 141.28 Approved laboratories.

For the purpose of determining compliance with § 141.21 through § 141.27, samples may be considered only if they have been analyzed by a laboratory approved by the State except that measurements for turbidity and free chlorine residual may be performed by any person acceptable to the State.

§ 141.29 Monitoring of consecutive public water systems.

When a public water system supplies water to one or more other public water systems, the State may modify the monitoring requirements imposed by this part to the extent that the interconnection of the sysems jusifies treating them as a single system for monitoring purposes. Any modified monitoring shall be conducted pursuant to a schedule specified by the State and concurred in by the Administrator of the U.S. Environmental Protection Agency.

Subpart D—Reporting, Public Notification and Record Keeping

§ 141.31 Reporting requirements.

(a) Except where a shorter reporting period is specified in this part, the supplier of water shall report to the State within 40 days following a test, measurement or analysis required to be made by this part, the results of that test, measurement or analysis.

(b) The supplier of water shall report to the State within 48 hours the failure to comply with any primary drinking water regulation (including failure to comply with monitoring requirements) set forth in this part.

(c) The supplier of water is not required to report analytical results to the State in cases where a State laboratory performs the analysis and reports the results to the State office which would normally receive such notification from the supplier.

§ 141.32 Public notification.

(a) If a community water system fails to comply with an applicable maximum contaminant level established in Subpart B, fails to comply with an applicable testing procedure established in Subpart C of this part, is granted a variance or an exemption from an applicable maximum contaminant level, fails to comply with the requirements of any schedule prescribed pursuant to a variance or exemption, or fails to perform any monitoring required pursuant to Section 1445 (a) of the Act, the supplier of water shall notify persons served by the system of the failure or grant by inclusion of a notice in the first set of water bills of the system issued after the failure or grant and in any event by written notice within three months. Such notice shall be repeated at least once every three months so long as the system's failure continues or the variance or exemption remains in effect. If the system issues water bills less frequently than quarterly, or does not issue water bills, the notice shall be made by or supplemented by another form of direct mail.

(b) If a community water system has failed to comply with an applicable maximum contaminant level, the supplier of water shall notify the public of such failure, in addition to the notification required by paragraph (a) of this section, as follows:

(1) By publication on not less than three consecutive days in a newspaper or newspapers of general circulation in the area served by the system. Such notice shall be completed within fourteen days after the supplier of water learns of the failure.

(2) By furnishing a copy of the notice to the radio and television stations serving the area served by the system. Such notice shall be furnished within seven days after the supplier of water learns of the failure.

(c) If the area served by a community water system is not served by a daily newspaper of general circulation, notification by newspaper required by paragraph (b) of this section shall instead be given by publication on three consecutive weeks in a weekly newspaper of general circulation serving the area. If no weekly or daily newspaper of general circulation serves the area, notice shall be given by posting the notice in post offices within the area served by the system.

(d) If a non-community water system fails to comply with an applicable maximum contaminant level established in Subpart B of this part, fails to comply with an applicable testing procedure established in Subpart C of this part, is granted a variance or an exemption from an applicable maximum contaminant level, fails to comply with the requirement of any schedule prescribed pursuant to a variance or exemption or fails to perform any monitoring required pursuant to Section 1445(a) of the Act, the supplier of water shall given notice of such failure or grant to the persons served by the system. The form and manner of such notice shall be prescribed by the State, and shall insure that the public using the system is adequately informed of the failure or grant.

(e) Notices given pursuant to this section shall be written in a manner reasonably designed to inform fully the users of the system. The notice shall be conspicuous and shall not use unduly technical language, unduly small print or other methods which would frustrate the purpose of the notice. The notice shall disclose all material facts regarding the subject including the nature of the problem and, when appropriate, a clear statement that a primary drinking water regulation has been violated and any preventive measures that should be taken by the public. Where appropriate, or where designated by the State, bilingual notice shall be given. Notices may include a balanced explanation of the significance or seriousness to the public health of the subject of the notice, a fair explanation of steps taken by the system to correct any problem and the results of any additional sampling.

(f) Notice to the public required by this section may be given by the State on behalf of the supplier of water.

(g) In any instance in which notification by mail is required by paragraph (a) of this section but notification by newspaper or to radio or television stations is not required by paragraph (b) of this section, the State may order the supplier of water to provide notification by newspaper and to radio and television stations when circumstances make more immediate or broader notice appropriate to protect the public health.

§ 141.33 Record maintenance.

Any owner or operator of a public water system subject to the provisions of this part shall retain on its premises or at a convenient location near its premises the following records:

(a) Records of bacteriological analyses made pursuant to this part shall be kept for not less than 5 years. Records of chemical analyses made pursuant to this part shall be kept for not less than 10 years. Actual laboratory reports may be kept, or data may be transferred to tabular summaries, provided that the following information is included:

(1) The date, place, and time of sampling, and the name of the person who collected the sample;

(2) Identification of the sample as to whether it was a routine distribution system sample, check sample, raw or process water sample or other special purpose sample;

(3) Date of analysis;

(4) Laboratory and person responsible for performing analysis;

(5) The analytical technique/method used; and

(6) The results of the analysis.

(b) Records of action taken by the system to correct violations of primary drinking water regulations shall be kept for a period not less than 3 years after the last action taken with respect to the particular violation involved.

(c) Copies of any written reports, summaries or communications relating to sanitary surveys of the system conducted by the system itself, by a private consultant, or by any local, State or Federal agency, shall be kept for a period not less than 10 years after completion of the sanitary survey involved.

(d) Records concerning a variance or exemption granted to the system shall be kept for a period ending not less than 5 years following the expiration of such variance or exemption.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

COLLECTION AND HANDLING OF DRINKING WATER SAMPLES

as applied in

DRINKING WATER TREATMENT FACILITIES
and in the
DISTRIBUTION SYSTEMS OF DRINKING WATER TREATMENT FACILITIES

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U. S. Environmental Protection Agency

Collection and Handling of Drinking Water Samples[1]

When the laboratory has been delegated responsibility for sample collecting, handling, and preservation, there must be strict adherence to correct sampling procedures, complete identification of the sample, and prompt transfer of the sample to the laboratory as described in "Standard Methods," 13th edition, section 450, p. 657-660.

The sample must be representative of the potable water system. The sampling program must include examination of the finished water at selected sites that systematically cover the distribution network.

Minimum sample frequency must be that specified in the National Interim Primary Drinking Water Regulations, 40 CFR 141.21.

The collector must be trained in sampling procedures and approved by the State regulatory authority or its delegated representative.

The water tap must be sampled after maintaining a steady flow for 2 or 3 minutes to clear service line. The tap is free of aerator, strainer, hose attachment, or water purification devices.

The sample volume must be a minimum of 100 ml. The sample bottle must be filled only to the shoulder to provide space for mixing.

The sample report form must be completed immediately after collection with location, date and time of collection, chlorine residual, collector's name, and remarks.

Sample bottles must be of at least 120 ml capacity, sterile plastic or hard glass, wide mouthed with stopper or plastic screw cap, and capable of withstanding repeated sterilization. Sodium thiosulfate (100 mg/l) is added to all sample bottles during preparation. As an example, 0.1 ml of a 10 percent solution is required in a 4-oz (120 ml) bottle.

Date and time of sample arrival must be added to the sample report form when sample is received in the laboratory.

State regulations relating to chain-of-custody, if required, must be followed in the field and in the laboratory.

Samples delivered by collectors to the laboratory must be analyzed on the day of collection.

Where it is necessary to send water samples by mail, bus, United Parcel Service, courier service, or private shipping, holding/transit time between sampling and analyses must not exceed 30 hours.

When possible, samples are refrigerated during transit and during storage in the laboratory (optional).

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[1] MINIMUM REQUIREMENTS except where indicated as OPTIONAL.

If the laboratory is required by State regulation to examine samples after 30 hours and up to 48 hours, the laboratory must indicate that the data may be invalid because of excessive delay before sample processing. Samples arriving after 48 hours shall be refused without exception and a new sample requested. (The problem of holding time is under investigation by EPA.)

At least one bottle per batch of sterilized sample bottles must be checked by adding approximately 25 ml of sterile LTB broth to each bottle. It must be incubated at 35 ± 0.5°C for 24 hours and checked for growth.

Dried glassware must be sterilized at a minimum of 170°C for 2 hours
Sample collection bottles (empty) ⎫
Individual glassware items ⎭ Autoclave sterilization at 121°C/30 minutes
Sample identified immediately after collection
Identification includes, water source, location, time and date of collection, and collector's name; insufficiently identified samples discarded
Chlorine residual where applicable

WATER MONITORING PROCEDURE: Collection and Handling of Drinking Water Samples

1. Analysis Objectives:

   Proper technique for the collection and handling of a sample for bacteriological examination taken from drinking water sources.

2. Brief Description of Analysis:

   After assembly of necessary equipment and travel to the sample site, the sample is collected in a manner which does not bias the sample and produces a representative sample. Samples are collected in a suitable, labeled, sterilized sample bottle which contains a chemical agent to inactivate the chlorine disinfectant in the sample collected.

   Controlled handling conditions maintain the integrity of the sample until its delivery to the laboratory within specified time limitations.

3. Applicability of this Procedure:

   Treatment of Interferences in Samples:

   This procedure includes directions for dechlorination of samples sufficient to act upon samples containing up to 15 mg/l of residual chlorine.

Source of Procedure: Standard Methods for the Examination of Water and Wastewater, 14th Ed., 1975.

WATER MONITORING PROCEDURE: Collection and Handling of Drinking Water Samples

General Description of Equipment and Supplies Used in the Procedure

A. Capital Equipment:

1. Autoclave, providing uniform temperatures up to, and including 121° C, equipped with an accurate thermometer, pressure gages, safety features, saturated steam power lines and capable of reaching required temperature within 30 minutes. Must perform sterilization cycle within 45 minutes.

   Alternately

   A pressure cooker can be substituted if:

   Efficient pressure gage
   Thermometer bulb 2.5 cm above water level.

2. Balance, 50 mg accuracy at a load of 2 or more grams. Should be clean and without corrosion; have good weights; and be calibrated annually.

3. Oven, drying and sterilizing. Capable of uniform temperatures and with suitable thermometer to register accurately in the range 160 - 180° C.

4. Refrigerator (at laboratory), set for range of 1.0 to 4.4° C.

5. Distillation Apparatus, Water. In order of preference, the systems are of stainless steel, quartz, vycor and pyrex glass. Tin-lined hardware is acceptable but because of maintenance problems is best avoided in preference to the above. Plumbing should be of stainless steel, pyrex or plastic PVC material. Storage reservoirs of stainless steel and dust protected. Produced water must be of suitable bacteriologic quality (test described in Standard Methods, 14th Edition, p. 887).

   Alternately

   Distilled water meeting this quality criteria can be purchased, eliminating the need for the distillation apparatus.

6. Washer, Glassware. Operate at 180° C during hot detergent cycle; hot rinse cycle of 6 to 12 successive washings; and final rinse of bacteriologically suitable distilled or deionized water. Produces clean sparkling glassware without spotting and meeting criterial of inhibitory residue test as described in Standard Methods, 14th Ed., p. 885.

B. Reusable Supplies:

1. Sample Bottle. Bacteriologically inert; resistant to sterilizing conditions; capacity at least 100 ml plus air space; containing dechlorinating agent if a sample containing chlorine is anticipated.

B. Reusable Supplies (Continued):

2. Sampler Device (unnecessary if bottle can be spigot or top filled which is the overwhelmingly usual occurrence). Line, wire, etc., if distance to sample water is sufficient to make line unwieldy or if sample water is reached with difficulty as through well covers, ports, etc. To avoid contamination of the water supply it is necessary to sterilize the whole assembly (including the sample bottle) and place in, for example, an aluminum foil bag which is opened when ready to sample.

3. Container, ice chest with cover.

C. Consumable Supplies:

1. Labels, clean and unused; non-smearing if wetted; sufficient size for needed information; can be attached securely to sample bottle.

2. Marking Device, non-smearing if wetted; permanent marking.

3. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$).

WATER MONITORING PROCEDURE: Collection and Handling of Drinking Water Samples

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Presampling Procedures			
1. Sample bottle inspection	1. Check sample bottles for acceptability.	1a. Provides at least 100 ml of sample volume and have a suitable head space above this volume.	V.A.1.1.1a (p. 2-16)
		1b. Suitable glass and/or plastic material.	V.A.1.1.1b (p. 2-16)
		1c. Bottles should not be chipped, cracked or otherwise damaged. No deposits or extensive glass scratches or etched surfaces can be tolerated. Bottle caps must not be cracked or otherwise damaged.	
		1d. Bottles should be sparkling clean and free from inhibitory or nutritive residues.	V.A.1.1.1d (p. 2-16)
2. Sodium thiosulfate solution	1. Weigh 10.0 grams of sodium thiosulfate.	1a. Used for dechlorination of samples. 1b. Use of trip balance accepted.	
	2. Dissolve in 50-60 ml distilled water.	2a. 100 ml graduated cylinder satisfactory.	
	3. Add distilled water to bring final volume to 100 ml.		
	4. Transfer to labeled bottle.	1a. Labeled as 10% sodium thiosulfate and stored in refrigerator. (Indicate date of preparation and who prepared). 1b. It is preferable to prepare less then 50 ml and sterilize the reagent to lessen the chance for contamination.	
3. Sample bottle preparation	1. Deliver 0.1 ml or .2 ml of 10% sodium thiosulfate solution to each sample bottle. (.1 ml to 4 ounce or 120 ml size and .2 ml to 6-8 ounce or 250 ml size).	1a. Use sterile 1 ml pipet if the reagent is sterile. 1b. Provides adequate sodium thiosulfate for neutralizing chlorine in sample. (100 mg/l). 1c. Return stock sodium thiosulfate solution to refrigerator. Solution must not be contaminated with microbial growth.	

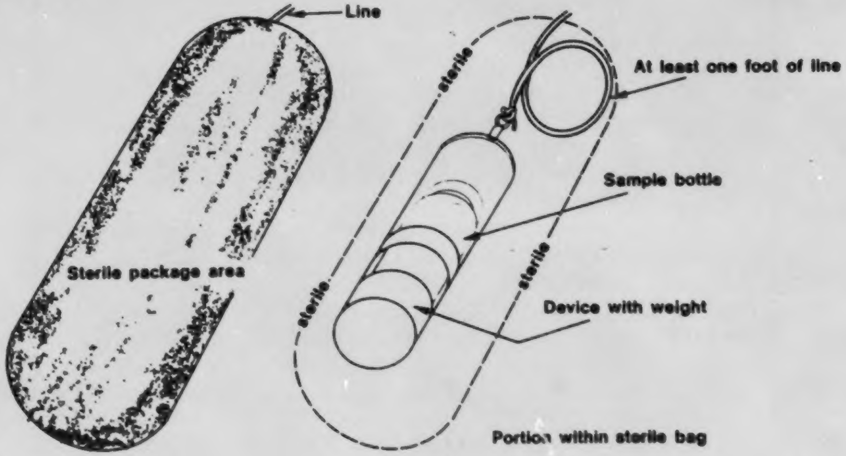
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Presampling Procedures (Continued)		1d. Do not exceed 0.4 ml of the 10% sodium thiosulfate to a bottle if a portion of the sample will be used for a Standard Plate Count.	
	2. Place cap on sample bottle.	2a. A string or paper wedge is placed between cap and neck of bottle when using ground glass stoppers to prevent "locking" at the interface.	
	3. Place Kraft paper or metal foil cover over bottle cap or stopper.	3a. Protects opening of sample bottle from accidental contamination. 3b. Cover (Hood) is not required for screw cap closures.	
	4. Sterilize sample bottles in sterilizing oven.	4a. Two hours at a minimum of 170° C. 4b. Steam sterilization (121° C for 30 minutes) can be used but oven sterilization is preferable.	V.A.3.4 (p. 2-17)
	5. Store sample bottles in clean, dry place until used.	5a. At least one bottle of each "batch" prepared should be checked for sterility and results entered in a quality control ledger.	VII.A.3.5 (p. 2-18)
4. Preparation of container for sample transportation and storage	1. Container inspected to be of sufficient size, leak proof, and have tight fitting cover.	1a. Size sufficient to hold all of required sample bottles. 1b. Use of this means of transportation and storage is OPTIONAL, but highly desirable if practiced. 1c. May be mandatory in tropical or subtropical areas where high surface temperatures prevail.	
	2. Layer bottom with ice just before departing for sampling points.	2a. To level about 1/2 of bottle height. 2b. Do not use dry ice.	
	3. Cover and assemble with other equipment.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Presampling Procedures (Continued)			
5. Inspection of sampling device	1. Check sampling device for condition.	1a. May be unnecessary if particular sampling run collects from devices which are easily accessible (spigots, taps, etc.). 1b. A number of suitable sampling devices are available and the function to (a) provide weight to allow the sampling device to reach a depth without drifting; (b) provide an anchoring point for the sterile bottle or chamber; (c) have a tripping mechanism to allow entry of sample to the collector; and (d) provide a means of lowering the device to depth and returned to surface. Check operation of each of these areas. Some types of samplers do not utilize a bottle but may function with sterile bulbs, bladders, etc. It will be necessary for the sampler to acquaint himself to the specific device being utilized at his facility.	
6. Assemble bottle labels	1. Check labels for acceptability.	1a. Must be clean and unused. 1b. Sufficient quantity for number of samples plus a few extra labels. 1c. Each label must have a means of attachment to sample bottles. Wire or cord is desirable and such attachments as scotch tape, electrical tape, etc. must be avoided as these are affected by moisture or water immersion. 1d. Labels can vary from that which is completely blank to a type which is required by the facility, agency, authority, etc. 1e. Minimum required data includes: * Sample location * Date and time of collection * Chlorine Residual	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Presampling Procedures (Continued)		1e. (Continued) *Collectors name (written out completely) *Laboratory reception (date/time) *Custody receipt (if necessary)	
7. Inspection of label marker	1. Check label marker for acceptability.	1a. Marker must be of a non-smearing permanent type. 1b. Marker is operable.	
B. Travel from Assembly Point to Sample Point	1. Proceed to initial sample point.	1a. Transport equipment with care. 1b. Upon arrival, recheck as to correctness of designated sampling point.	VII.B.1.1b (p. 2-18)
	2. Prepare sample station for collection of sample. (If special collection situation. See C.2).	2a. Remove manholes, ports, access panels, etc., if necessary. 2b. Note safety hazards at site. It is necessary to have a partner if potentially hazardous conditions can result in injury or death if another person is not available for help.	
C. Sample Collection Procedures			
1. Spigot or tap	1. Prepare spigot for sampling.	1a. Must not have strainers, screens, aerators, etc., which can harbor bacteria or particulate matter. Remove these attachments. 1b. Spigot must not have leaks past gaskets, washers, etc., and these spigots must be eliminated as sampling points.	VII.C.1.1 (p. 2-18)
	2. Flush spigot.	2a. Must have direct main connection and be representative of system 2b. Full flow flush for 2-3 minutes or enough to clear service line. 2c. Hand pump activated taps should be flushed for 5 minutes.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Sample Collection Procedures (Continued)	3. Adjust spigot for steady flow.	3a. Avoids "scouring" of system by vigorous flow which could cause sampling of nonrepresentative bacterial or material particles.	
	4. Remove hood and cap from sample bottle (glass type) or screw cap from other types.	4a. Remove hood and cap as unit. 4b. Discard slip of paper or string which is between cap and bottle interface. 4c. Protect units from contamination. Usual method is to hold cap in left hand (if right-handed) and have bottle in right hand.	
	5. Let sample run into bottle.	5a. No rinsing of bottle. Especially important if bottle contains sodium thiosulfate. 5b. Fill about 3/4 full so that a mixing space is available for thorough sample mixing prior to laboratory operations. (At least 2.5 cm of air space required). 5c. Sample must not be decanted (completely filled and then a portion discarded to give the required air space.)	
	6. Replace cap and hood or screw-cap on bottle.	6a. Secure closure but not excessively tightened or wedged on bottle.	
	7. Label bottle with tag.	7a. Fill all items demanded by drinking water requirements and others as needed by local authorities.	See A.6
	8. Place bottle on ice in ice chest (optional).	8a. Do not immerse bottle in excessive water volume. Remove excessive water if present in chest. 8b. Cover chest. 8c. Do not use dry ice as freezing is detrimental to sample. 8d. Do not composite sample (mix different portions of collected sample from different or the same sample point).	IMPORTANT VII.C.1.8 (p. 2-18)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Sample Collection Procedures (Continued)			
2. Special collection situation	1. Remove sample device from packaging.	1a. Packaging (Kraft paper, aluminum foil, etc.) preserves sterility of device and sample bottle 1b. Remove carefully to avoid contaminating device.	See Sample Device under Reusable Equipment Also B.2



WATER MONITORING PROCEDURE: Collection and Handling of Drinking Water Samples

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Sample Collection Procedures (Continued)		1c. Handle only beyond one foot of line to avoid contaminating water supply.	
	2. Lower device until under water about 6 inches.	2a. Avoid hitting sides of well, reservoir, etc. 2b. Under water to avoid collecting surface scum, debris, etc.	
	3. Activate device to obtain sample.	3a. Many methods available to activate device (electrical; messenger [brass plug with center hole dropped down line]; another line pulled; etc.).	
	4. Retrieve sample from device.	4a. Pull up device carefully without touching sides of well; tank; etc. 4b. Remove sample bottle from device with cap, if of this design, and secure bottle. 4c. Some devices may not have a cap with the bottle and may necessitate transfer of sample to another sterile container. Shake well before transfer; treat asceptically (without contaminating); and be sure that new bottle has at least 2.5 cm of head space when complete sample is contained.	
	5. Label bottle with tag.	5a. Fill all items demanded by drinking water requirements and others as needed by local authorities.	
	6. Place bottle on ice in ice chest (optional).	6a. Precautions as in C.1.8.8a-d.	VII.C.1.8 (p. 2-18 & 2-19)
D. Sample Handling	1. Transport sample to laboratory.	1a. Any of the transport modes previously discussed. 1b. Must be in the hands of the laboratory personnel within 30 hours (elapsed time from sample collection to start of analysis).	VII.D.1.1b (p. 2-19)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Sample Handling (Continued)		1c. If sample is delivered directly by a sampler to the laboratory, it must be processed for analysis on the same day. 1d. Observe log-in procedures as established by laboratory and custody receipt procedures observed for samples which may have legal implications.	

WATER MONITORING PROCEDURE: Collection and Handling of Drinking Water Samples

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
VII*	Field & Laboratory Analyses
VIII	Safety
IX	Records and Reports

Training guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C	The sampler must be trained and approved by the appropriate State agency or its designated representative.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1.1a	A sample bottle size which will provide the required 100 ml volume (minimum sample volume) as well as at least 2.5 cm of air space above this volume for sample mixing is mandatory.	
A.1.1.1b	Sample bottles must be composed of a material which is non-toxic to bacteria, resistant to solvent action of sample, and capable of being repeatedly sterilized without leakage occurring.	
	If glass-stoppered bottles are used, a strip of paper or string should be placed in the neck of the bottle before placing the stopper in place in preparation for sterilization. This prevents the glass stopper from "freezing" in place during sterilization. The paper strip is discarded at the time of sample collection.	
	Various plastics (polypropylene, Nalgene, etc.) have been found to meet the specifications above, and, closures of the screw cap type are acceptable provided they are, and remain, non-toxic to the sample and provide a tight closure. A test is described to check new caps for toxicity (see reference).	EPA-670/9-75-006 Handbook for Evaluating Water Bacteriological Laboratories
	It is wise to purchase plastic bottles and caps of the same material to preclude immediate or delayed leakage problems.	
A.1.1.1d	Bottles can be checked for bacteriostatic or inhibitory residues by a bacteriological test procedure which, like the distilled water suitability test, should be undertaken only by professional bacteriologists or in laboratories where this test is done on a regular basis.	Std. Meth: 14:885
	All glassware (bottles, pipets, graduates, etc.) should be thoroughly cleaned with a suitable detergent and hot water. Following several rinses of hot tap water, several successive rinses with a bacteriologically suitable deionized or distilled water will produce a suitable bottle whether this operation is accomplished by machine or hand washing.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.3.4	Use of sterilizing oven ensures the drying of the thiosulfate reagent on the bottom of the bottle which will be visible as a thin white film. Items should be inserted into a cool oven and not removed until cooled after the sterilizing cycle. Whichever method of sterilizing is used (dry heat or steam), complete records are to be maintained in a ledger which shows date; temperatures, time of cycle; and laboratory workers name.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.3.5	To check the sterility of a sterilized "batch" of sample bottles, remove one or more bottles from the group of bottles which were unit sterilized and test as follows: 1. Aseptically pipet (sterile technique) approximately 25 mls of sterile LLSTB (lactose lauryl sulfate tryptose broth) into the sample bottle. 2. Incubate for 24 hours at 35° C. 3. Check for results: A. Growth (turbidity) is unsatisfactory and indicates lactose fermenters as contaminants. B. No growth indicates acceptability and the bottle is sterile as regards to lactose fermenters. 4. Record results in a bound quality control ledger. 5. Discard any unsatisfactory batches (positives) and investigate source of problem.	EPA-670/9-75-006 Handbook for Evaluating Water Bacteriological Laboratories
B.1.1b	Sampling frequency and locations are stipulated under existing governmental requlations. Establishment and changes of the above are joint functions of the Certifying Authority (Regional EPA Water Supply Representative); Reporting Agency (State engineering program); and the Utility (local administration).	NIP DWR 40 CFR 141.21
C.1.1	Spigot or tap does not have to be "flamed" or heated as these treatments have been found to be of no consequence regarding bacteriological testing and can, in addition, cause damage to valve components.	
C.1.8	Icing a sample prior to delivering it to the analyzing laboratory is the most desirable holding method. It is, however, acceptable to handle it in a number of ways:	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.1.8 (Continued)	1. If less than an hour will elapse between collection and laboratory acceptance, icing is not an important factor in handling. 2. Although not a desirable practice, it is acceptable to transport a sample, uniced, in a number of modes (mail; bus; UPS; courier service; private shipping; etc.) as long as the transit time is within limitations. Another optional choice to the icing mode of transportation is the use of thermos-type, insulated, and sterilizable container.	
D.1.1b	Current regulations specify this 30 hour limitation period beyond which the sample is unacceptable for data validity. It is, however, a possibility that the responsible State Agency may require the laboratory to run the sample if it is received after this period. If more than 30 hours, but less than a 48 hour, period has elapsed (from collection time to initiation of laboratory procedures), the laboratory may run the sample with the requirement that the data is indicated to be possibly invalid. In the event that greater than 48 hours of holding time has elapsed, the laboratory must refuse the sample as unsuitable for analysis.	
	This outline was prepared by: Rocco Russomanno, Microbiologist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

COLIFORM TEST BY THE MPN METHOD FOR DRINKING WATER

as applied in

DRINKING WATER TREATMENT FACILITIES
and in the
DISTRIBUTION SYSTEMS OF DRINKING WATER TREATMENT FACILITIES

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

Coliform Test by the MPN Method for Drinking Water[1]

MPN media removed and cooled as soon as possible after sterilization and stored in cool dark place (optional).

Tube broth media and reagents sterilized at 121°C 12 to 15 minutes with tubes and flasks packed loosely in baskets or racks for uniform heating and cooling.

Laboratory pure water must be used; dissolution of the media must be completed before dispensing to culture tubes or bottles.

Lauryl Tryptose Broth (Lactose broth is not permitted.)	Single strength composition, 35.6 g per liter pure water Single strength pH 6.8 ± 0.2; double strength pH 6.7 ± 0.2 Not less than 10 ml per tube Media made to result in single strength after addition of sample portions

Brilliant Green Lactose Bile Broth - Medium composition 40 g per liter pure water. Final pH 7.2 ± 0.2

Media stored at low temperatures are incubated overnight prior to use and tubes with air bubbles discarded
Media protected from sunlight
MPN tube media with loose-fitting caps used in less than 1 week
Tube media in screw-capped tubes held no longer than 3 months

Presumptive Test

Five standard portions, either 10 or 100 ml
Sample shaken vigorously immediately before test
Tubes incubated at 35° ± 0.5°C for 24 ± 2 hours
Examined for gas (any gas bubble indicates positive test)
Tubes that are gas-positive within 24 hours submitted promptly to confirm test
Negative tubes returned to incubator and examined for gas within 48 ± 3 hours positives submitted to confirm test
Public water supply samples with heavy growth and no gas production confirmed for presence of suppressed coliforms
Adjusted count reported based upon confirmation
Adequate test labeling and tube dilution coding (optional)

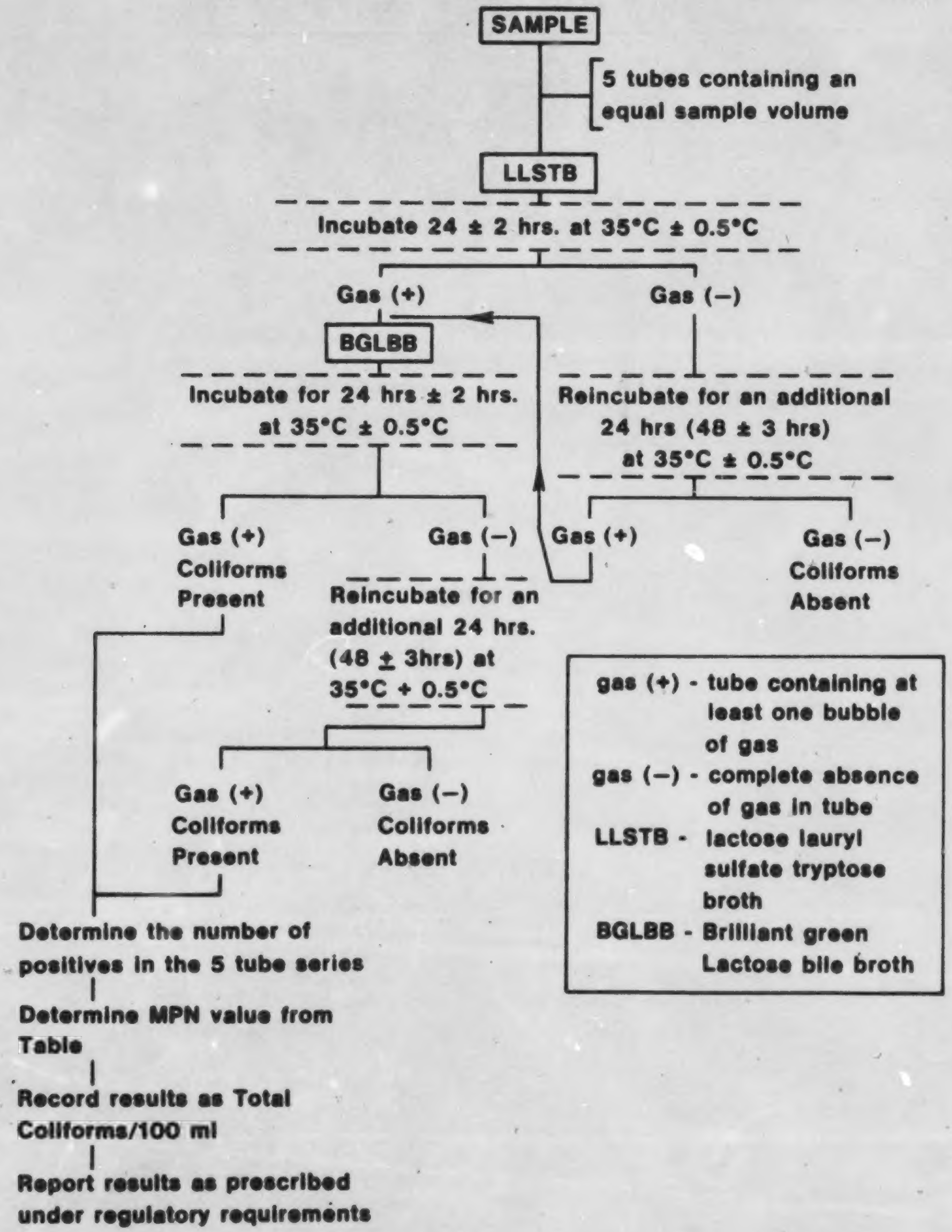
Confirmed Test

Presumptive positive tube gently shaken or mixed by rotating
One loopful or one dip of applicator transferred from presumptive tube to BGLB. Transfer device sterile.
Incubated at 35°C ± 0.5°; checked within 24 hours for gas production
Positive confirmed tube results recorded; negative tubes reincubated and read within 48 ± 3 hours
Unsatisfactory sample defined as three or more positive confirmed tubes

[1] MINIMUM REQUIREMENT except where indicated as OPTIONAL.

Confirmed MPN test on problem supplies: If the laboratory has elected to use the MPN test on water supplies that have a continued history of confluent growth or TNTC with the MF procedure, all presumptive tubes with heavy growth without gas production should be submitted to the confirmed MPN test to check for the suppression of coliforms. A count is adjusted based upon confirmation and a new sample requested. This procedure should be carried out on one sample from each problem water supply once every 3 months.

DRINKING WATER COLIFORM TEST
MPN METHOD FLOW SHEET


SAMPLE
5 tubes containing an equal sample volume
LLSTB
Incubate 24 ± 2 hrs. at 35°C ± 0.5°C
Gas (+)
Gas (–)
BGLBB
Incubate for 24 hrs ± 2 hrs. at 35°C ± 0.5°C
Reincubate for an additional 24 hrs (48 ± 3 hrs) at 35°C ± 0.5°C
Gas (+) Coliforms Present
Gas (–)
Gas (+)
Gas (–) Coliforms Absent
Reincubate for an additional 24 hrs. (48 ± 3hrs) at 35°C + 0.5°C
Gas (+) Coliforms Present
Gas (–) Coliforms Absent
Determine the number of positives in the 5 tube series
Determine MPN value from Table
Record results as Total Coliforms/100 ml
Report results as prescribed under regulatory requirements

gas (+) -	tube containing at least one bubble of gas
gas (–) -	complete absence of gas in tube
LLSTB -	lactose lauryl sulfate tryptose broth
BGLBB -	Brilliant green Lactose bile broth

WATER MONITORING PROCEDURE: Coliform Test by the MPN Method for Drinking Water

1. Analysis Objectives:

   a. In water treatment plant quality control, the objective of the test is to determine if the effluent quality is in compliance with bacteriological requirements as prescribed in the Federal Drinking Water Standards.

   b. In distribution network and individual consumer tapping locations, the test determines compliance with bacteriological requirements with the above mentioned standards.

2. Brief Description of Analysis:

   Five standard portions of either 100 ml or 10 ml are inoculated from a drinking water sample into Lactose Lauryl Sulfate Tryptose broth fermentation tubes (LLSTB) and incubated at 35°C ± 0.5°C. After 24 hours and again at 48 hours, the LLSTB tube cultures are examined and results recorded for gas production. Cultures showing gas production are transferred at each examination interval to BGLBB fermentation tubes and incubated at 35°C ± 0.5°C. BGLBB tubes are examined at 24 and 48 hour intervals for presence of gas and those showing gas are considered gas (+) and containing coliforms while those completely without gas as gas (-) or not containing coliforms.

   At the end of the overall incubation period, individual tubes are summarized as positive or negative and the number positive for the five tubes is obtained. A table of Most Probable Numbers (MPN) is used to determine the MPN value which is given as coliforms per 100 ml. This result is reported or handled as prescribed under regulatory requirements.

3. Applicability of this Procedure:

   a. The range of coliform concentrations:

If this sample volume is used	These ranges of coliforms are covered
100 ml	< .22 to > 1.6
10 ml	< 2.2 to > 16

   b. Pretreatment of Samples: In accordance with Standard Methods, 14th ed. (p. 904)

This procedure conforms to the Standard Total Coliform MPN Tests as described in Standard Methods for the Examination of Water and Wastewater, 14th ed. (1975). p. 914

WATER MONITORING PROCEDURE: Coliform Test by the MPN Method for Drinking Water

Equipment and Supply Requirements

A. Capital Equipment:

Autoclave, providing uniform temperatures up to and including 121°C, equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperature within 30 minutes.

Balance, 0.1 g sensitivity at load of 150 g

Air incubator to operate at 35°C ± 0.5°C

Oven, *hot-air sterilizing, to give uniform temperatures and with suitable thermometer to register accurately in range of 160-180°C

pH meter, accurate to at least 0.1 pH unit, with standard pH reference solution(s)

Water distillation apparatus (glass or block tin), or source of distilled water suitable for bacteriological operations.

B. Reusable Supplies:

Apron or coat suitable for laboratory

Baskets, wire for discarded cultures

Bottles, sample*, preferred characteristics being 250 ml (6-8 oz.), wide mouth, glass stopper

Bottle, squeeze type, with disinfecting solution

Burner, gas, Bunsen burner type

Cans, pipet, aluminum or steel; not copper (If plastic, or other type of prepackaged disposable pipets are used, this item is unnecessary.)

Metal caps* to fit 25 x 150 mm and 18 x 150 mm culture tubes

Pan, to receive discarded contaminated pipets and glassware (must contain disinfectant before use)

Inoculation loop, 3 mm diameter loop of nichrome of platinum-iridium wire, 26 B&S gauge, in holder

Pipets*, 10 ml, Mohr type preferred, sterile, cotton plugged, glass or disposable plastic

Racks, culture type*, having at least 5 openings capable of accepting tubes at least 20 mm in diameter

Equipment and Supply Requirements (Continued)

Sponge, for cleaning desk top

Tubes, culture*, 150 x 25 mm and 150 x 18 mm

Tubes, fermentation*, 75 x 10 mm vials to be inverted in culture tubes

C. Consumable (must be replaced when stocks get low):

Distilled water, suitable for bacteriological cultures (note distillation apparatus required in capital equipment)

BGLBB (Brilliant Green Lactose Bile Broth), dehydrated (recommend purchase of 1/4-lb. units)

Lactose Lauryl Sulfate Tryptose Broth, dehydrated (recommend purchase of 1-lb. units)

Potassium Dihydrogen Phosphate (KH_2PO_4) (recommend purchase of 1/4 lb. units)

Disinfectant, for bench tops. (Use household bleach solution prepared according to instructions on bottle)

Wax pencils (recommend soft wax equivalent to Blaisdell 169T)

Sodium Thiosulfate ($Na_2S_2O_3 \cdot 5\ H_2O$)

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*Items marked are needed in quantities or require size or space allowances which cannot be specified here, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of "Standard Methods for the Examination of Water and Wastewater."

WATER MONITORING PROCEDURE: Coliform Test by the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures		Aa. All pretest procedures completed before starting other first-day procedures.	
1. 35°C Incubator Set-up, Adjustment	1. Place 35°C incubator in permanent location.	1a. Out of drafts or places where it will be in direct sunlight part of the day. 1b. Location convenient to laboratory bench. 1c. Convenient source of electric power.	V.A.1 V.A.1.1 (p. 31)
	2. Install thermometer.	2a. Thermometer functions at least in 30°-40°C range and have intervals of 0.5° or less indicated. Meets NBS standards. 2b. Location should be central in incubator. 2c. Mercury bulb thermometer should be fitted with cork or rubber stopper and mounted in small bottle filled with liquid (glycerine, water, or mineral oil).	V.A.1.2 (p. 31)
	3. Install shallow pan of water in bottom of incubator.	3a. In most laboratory incubators a pan having about 1 square foot area, with water about 1 inch deep, is satisfactory. 3b. Maintains condition of saturated relative humidity, required in bacteriological incubator. 3c. Requires daily check, with addition of water as necessary, to keep water in pan at all times.	V.A.1.3 (p. 31)
	4. Connect incubator to electric power source.	4a. Many incubators have pilot light to indicate power turned on.	
	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for method of temperature adjustment. 5b. Operation must be at 35 ± 0.5°C. 5c. Allow about 1 hour between adjustments.	V.A.1.5 (p. 31)
	6. Operate bacteriological incubator continuously.	6a. Requires daily check with written temperature record, with adjustment and water addition as necessary.	V.A.1.6 (p. 31)

WATER MONITORING PROCEDURE: Coliform Test by the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)			
2. Oven, Sterilizer, Set-up	1. Place oven sterilizer in permanent location.	1a. Convenient to source of electric power; usually on table or bench.	V.A.2.1-5 (p. 31)
	2. Install thermometer.	2a. Should indicate the 150°-180°C range, be accurate within this interval, and be marked in 1.0 degree intervals. Thermometer bulb is within a cylinder filled with a fine sand and positioned on the center shelf of the chamber.	
	3. Connect oven sterilizer to power source and turn on.	3a. Usually has pilot light to indicate power on.	
	4. Adjust temperature to stabilize at required temperature.	4a. Operated as near to 170°C as possible; not lower than 160 nor higher than 180°C. Check to verify that the 170°C temperature is reached and is maintained within ± 10°C for a 2-hour period.	
	5. Operate oven sterilizer only when needed. Turn off when not in use.	5a. Turned ON in advance of need to permit reaching required temperature before introducing material to be sterilized. 5b. Oven sterilizer used to sterilize dry glassware, metal objects. 5c. Oven sterilizer not used with culture media, solution, plastics, rubber objects, or with anything containing or including these. 5d. Paper-wrapped glass pipets may be sterilized in oven sterilizer.	
3. Autoclave Set-up	1. Install and operate autoclave according to manufacturer's instructions.	1a. Autoclaves extremely variable in design and operation; also, potentially dangerous. 1b. Used to sterilize objects made of, or including liquids, rubber, culture media. 1c. Glassware may be autoclave sterilized but must be dried afterward.	V.A.3.1 (p. 32)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)		1d. Most plastics not sterilized in autoclave; plastics usually require chemical sterilizers. 1e. Autoclave usually operated at 121°C for 15 min. 1f. Sterilized media must be removed from autoclave as soon as possible after autoclave is reopened.	
4. Water Distillation Equipment	1. Install and operate in accordance with manufacturer's instructions.	1a. Must produce distilled water meeting quality requirements for bacteriological tests.	V.A.4.1-2 (p. 32)
	2. Operate continuously or intermittently as required to maintain adequate supplies of distilled water.	2a. Reserve supplies kept in borosilicate glass carboys or in plastic carboys made of material which will not dissolve substances which will affect growth of bacteria. 2b. Same distillation apparatus used for bacteriological purposes may be used for chemical reagents.	
5. pH Meter	1. Have unit available and operate in accordance with procedures described in other lab procedures.	1a. Unit for pH check on finished culture media. 1b. Used in preparation of stock solution of potassium dihydrogen phosphate.	V.A.5.1 (p. 33)
6. Glassware	1. Wash all glassware in hot detergent solution;	1a. Nontoxic detergent. 1b. Be sure all contents and markings are washed away.	V.A.6.1-4a (p. 33)
	2. Rinse at least once in hot tap water;		
	3. Rinse in distilled water, at least 6 successive times, and,		
	4. Dry in air.	4a. No visible spots or scum; glass should be clean, and sparkling. 4b. Glassware suitable for use in bacteriological operations.	V.A.6.4b (p. 33)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)			
7. Sodium Thiosulfate Solution	1. Weigh 10.0 grams of sodium thiosulfate.	1a. Used for dechlorination of samples. 1b. Use of trip balance accepted.	
	2. Dissolve in 50-60 ml distilled water.	2a. 100 ml graduated cylinder satisfactory.	
	3. Add distilled water to bring final volume to 100 ml.		
	4. Transfer to labeled bottle.	4a. Labeled as 10% sodium thiosulfate and stored in refrigerator.	
8. Sample Bottle Preparation	1. Deliver 0.1 ml or .2 ml of 10% sodium thiosulfate solution to each sample bottle. (.1 ml to 4 ounce or 120 ml size and .2 ml to 6-8 ounce or 250 ml size)	1a. Use 1 ml pipet. 1b. Provides adequate sodium thiosulfate for neutralizing chlorine in sample. 1c. Return stock sodium thiosulfate solution to refrigerator.	V.A.8.1-6 (p. 33)
	2. Place cover on sample bottle.	2a. Use 1 ml pipet. 2b. Provides adequate EDTA chelating agent for metals in sample.	
	3. Place paper or metal foil cover over bottle cap or stopper.	3a. Protects opening of sample bottle from accidental contamination.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	4. Sterilize sample bottles in sterilizing oven.	5a. One hour at 170°C. (See A.2)	
	5. Store sample bottles in clean, dry place until used.		
9. Pipet Preparation	1. Inspect pipets to be prepared for use; discard and destroy all having chipped or cracked tips or tops.	1a. Cleanliness of pipet must be equivalent to glassware.	
	2. Insert plug of non-absorbent cotton into mouthpiece of each clean, dry pipet.	2a. For protection of user when pipetting sample. 2b. Cotton plug must be tight enough to prevent easy removal, either by the pipetting action or by handling, and yet loose enough to permit easy air movement through the plug.	V.A.9.1-6 (p. 33)
	3. Place a layer of glass wool or several layers of paper padding in bottom of pipet can.	3a. For protection of pipet delivery tips.	
	4. Place 18-24 pipets in each pipet can, delivery tip down.	4a. Orientation permits removal of sterile pipets from can without contamination by user.	
	5. Sterilize cans of pipets in oven.	5a. One hour at 170°C (See A.2 of procedures).	
	6. Store cans in clean, dry place until used. Mark cans as 10 ml sterile pipets.	6a. Laboratory cabinet or drawer recommended.	

WATER MONITORING PROCEDURE: Coliform Test by the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	7. When can of pipets is opened for first use, pass the exposed ends of the pipets through flame, slowly.	7a. Burns off excess cotton sticking out of pipet mouthpiece. 7b. Cover kept on can at all times except when samples are being inoculated.	V.A.9.7 (p. 34)
10. Preparation of Lactose Lauryl Sulfate Tryptose Fermentation Broth (LLSTB)	1. Weigh 53.4 grams of dehydrated Lactose Lauryl Sulfate Tryptose Broth. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media take moisture out of air; can become caked. 1b. Caked media unsatisfactory; should be discarded.	
	2. Dissolve in 1 liter distilled water.	2a. Gentle heat (no boiling) if necessary to complete dissolving medium. Usually a vigorous agitation will completely dissolve the medium.	
	3. Place 20.5 ml of the solution of prepared LLSTB in each culture tube.	3a. Use 150 x 25 mm tubes. 3b. 25 ml pipet, automatic pipetter, or funnel hose and pinchcock assembly are acceptable. 3c. Accuracy of delivery: ± 0.5 ml. 3d. Approximately 45 tubes will be necessary. This will suffice for 9 tests based upon procedures of this WMP (Water Monitoring Procedure).	V.A.10.3b (p. 34)
	4. Insert one fermentation vial into each tube of medium, open end down.	4a. Tubes and vials previously washed as indicated (V.6.1-4). 4b. Use 75 x 10 mm tubes.	
	5. Place tube cap on each tube of culture medium.	5a. After all tubes have been filled and have individual vial.	
	6. Sterilize in autoclave.	6a. Within 1 hour after medium prepared. 6b. Sterilization at 121°C for 15 minutes. 6c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" mode of steam removal.	

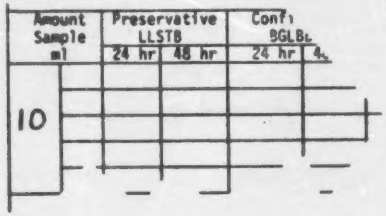
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	7. Cool medium to room temperature.	7a. Medium ready for use when cool and individual vials are completely filled with fluid. No bubbles must be present.	
	8. Check pH of finished medium.	8a. Should be 6.7-6.9.	
	9. If final pH not satisfactory, discard medium and prepare new batch with pH adjustment before sterilization.	9a. pH value ordinarily drops about 0.2 pH unit.	
	10. Store medium in cool, dark place.	10a. Not in refrigerator. Usually in laboratory cabinet in darkness. 10b. May be stored up to 1 week if evaporation not more than 10% in loose fitting capped tubes. With screw-capped tubes, it should be held no longer than 3 months.	
11. Preparation of Brilliant Green Lactose Bile Broth (BGLBB)	1. Weigh 40.0 grams of dehydrated Brilliant Green Lactose Bile Broth. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media takes moisture out of air; can become caked. 1b. Caked media unsatisfactory; should be discarded.	
	2. Dissolve in 1 liter of distilled water.	2a. Gentle heat (no boiling) if necessary to complete dissolving medium. Usually a vigorous agitation will completely dissolve the medium.	
	3. Place 10.5 ml of the solution of prepared BGLBB in each culture tube.	3a. Use 150 x 18 mm tubes. 3b. A 25 ml pipet, automatic pipetter or funnel hose and pinchcock assembly are acceptable. 3c. Accuracy of delivery ± 0.5 ml. 3d. Approximately 90 tubes will be necessary.	V.A.10.3b (p. 34)

WATER MONITORING PROCEDURE: Coliform Test by the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	4. Insert one fermentation vial into each tube of medium, open end down.	4a. Tubes and vials previously washed as indicated (A.6.1-4). 4b. Use 75 x 10 mm tubes.	
	5. Place cap on each tube of culture medium.	5a. After all tubes have been filled and have individual vial.	
	6. Sterilized in autoclave.	6a. Within 1 hour after medium prepared. 6b. Sterilization at 121°C for 15 minutes. 6c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" mode of steam removal.	
	7. Cool medium to room temperature.	7a. Medium ready for use when cool and individual vials are completely filled with fluid. No bubbles must be present.	
	8. Check pH of finished medium.	8a. Should be 7.1-7.3.	
	9. If final pH is not satisfactory, discard medium and prepare new batch with pH adjustment before sterilization.	9a. pH value ordinarily drops about 0.2 pH unit.	
	10. Store medium in cool, dark place.	10a. Not in refrigerator. Usually in laboratory cabinet in darkness. 10b. May be stored up to 1 week if evaporation not more than 10% in loose-fitting capped tubes. With screw-capped tubes, it should be held no longer than 3 months.	
12. Final Equipment and Supply Check	1. Check to be sure that all equipment and supplies, solutions, and prepared media are ready before starting sample examination.	1a. Check general list of equipment and supplies. 1b. Each test requires: 5 tubes LLSTB 0-5 tubes of BGLBB 1 sample bottle 1-10 ml pipet	

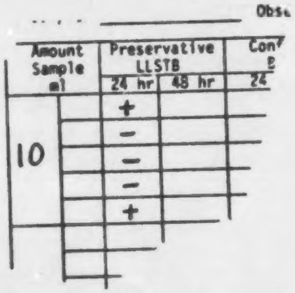
WATER MONITORING PROCEDURE: Coliform Test by the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	2. Make preparations or adjustments as necessary before starting test.		
B. First-Day Procedures			
1. Equipment Maintenance	1. Check, record, and adjust incubator temperature.	1a. See A.1.1-6.	
	2. Add water to pan in incubator as necessary.		
2. Sample Collection	1. Collect sample.	1a. Locations as selected by requirements. 1b. Sampling methods as described in Standard Methods.	
	2. Record sampling information.	2a. Most organizations have sample tag of some type which includes such information as date, time, place of sampling, name of sample collector, and other information as may be required.	
	3. Transport sample to laboratory.	3a. Taken to laboratory without delay. 3b. Samples preferably iced if delay of starting sample test is greater than one hour. No more than 30 hours of transportation time is allowed.	
3. Preparation of Laboratory Data Sheet	1. Fill in data sheet to show sample information.	1a. Needed information should be on sample collection tag. 1b. Most data sheets show at least source, date, time of collection, name of sampler, name of analyst, laboratory sample number assigned.	VII.B.3.1 (p. 35)
	2. Select sample inoculation volumes.	2a. For purposes of this WMP (Water Monitoring Procedure), sample volumes of 10 ml per tube in a series of 5 tubes is required.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-Day Procedures (Continued)		2b. Workers desiring 100 ml portions for this test should consult Standard Methods for required modifications (medium strength, interpretation of results, etc.)	
	3. Enter information in laboratory data sheet to show sample inoculation volume for 5 tubes.	3a. Recommend showing sample inoculation volumes in ml or decimal amounts.  Amount Sample ml Preservative LLSTB 24 hr 48 hr Conf BGLB 24 hr 4 10	
4. Lab Bench Disinfection	1. Disinfect laboratory bench; wipe dry.	1a. Sponge and disinfectant; paper toweling.	
5. Assembly of Culture Medium	1. Place 5 tubes of Lactose Lauryl Sulfate Tryptose Broth (LLSTB) in culture tube rack.		
	2. Label test set-up.	2a. First tube or rack can be labeled. 2b. Prevents confusion if a number of tests are being processed.	
6. Sample Inoculation	1. Shake sample vigorously.	1a. At least 25 shakes over space of at least 1 foot in 10 seconds or less.	I.B.6.1 (p. 28)
	2. Deliver 10 ml of sample per tube of LLSTB.	2a. Use sterile 10 ml pipet.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-Day Procedures (Continued)			
7. Incubation	1. After completion of sample inoculation into LLSTB, shake rack of cultures gently.	1a. Mixes sample with culture medium. 1b. Avoid Shaking air into fermentation vials.	
	2. Place rack of cultures in incubator.	2a. Twenty-four hours ± 2 hours at 35 ± 0.5°C.	
8. Processing Used Glassware	1. Drain sample bottle and pipet into sink.	1a. Sterilization unnecessary.	
	2. Wash and dry bottle and pipet.	2a. Meets original cleanliness requirements of glassware. 2b. Glassware ready for reuse.	
9. Lab Bench Disinfection	1. Disinfect laboratory bench top; wipe dry.	1a. Sponge, disinfectant, paper toweling.	
C. Twenty-four Hour Procedures			
1. Equipment Maintenance	1. Check, record, and adjust incubator temperature.	1a. See A.1.1-6.	
	2. Add water to pan in incubator as necessary.		
2. Disinfection	1. Disinfect laboratory bench top; wipe dry.	1a. See B.4.1.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Twenty-four Hour Procedures (Continued)			
3. Reading and Recording of Results	1. Remove rack of culture from incubator to lab bench.		
	2. Shake culture rack gently.	2a. Hastens release of gas in supersaturated cultures. 2b. Must not shake air into fermentation vials.	
	3. Examine each tube for gas production and record results on data sheet.	3a. If present, gas will be trapped in the fermentation vial. 3b. Gas in any quantity is a positive test. 3c. Vials with no gas are a negative test. 3d. Each result appears on line corresponding with the tube label. 3e. All results appear under the "24" of the LLSTB column. 3f. Plus sign (+) means a gas-positive tube. 3g. Minus sign (-) means a gas-negative tube. 3h. Assume, for instructional purposes, that the following recordings result:	III.C.3.3 (p. 30)



Obs

Amount Sample ml		Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Con 24
10		+		
		−		
		−		
		−		
		+		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Twenty-four Hour Procedures (Continued)			
4. Transfers	1. Obtain required number of BGLBB tubes.	1a. One tube for each LLSTB gas-positive tube. 1b. In our example test it is necessary to have two BGLBB tubes. 1c. Observe BGLBB tubes for sterility (no growth or turbidity) and completely filled fermentation vial (no gas in vial).	
	2. Transfer each gas-positive tube of LLSTB to a tube of BGLBB.	2a. 3-mm inoculation loop. 2b. Loop flame-sterilized before use and between successive transfers. 2c. One loopful per transfer. 2d. Place inoculated BGLBB tube into hole of rack previously occupied by the LLSTB tube from which the transfer was made. 2e. Place positive LLSTB tube into discard area after transfer is made. All discard tubes are to be sterilized prior to cleaning and reuse of caps and tubes. 2f. Negative (no gas) LLSTB tubes remain untouched in their rack position.	VII.C.4.2 (p. 36)
	3. Return rack of tubes containing the negative LLSTB tubes and the freshly inoculated BGLBB tubes to incubator.	3a. An additional 24 ± 2 hours at 35° ± 0.5°C.	
5. Processing Discarded Cultures	1. Sterilize discarded LLSTB tubes.	1a. Autoclave: 15 minutes at 121°C.	
	2. Remove all labels from culture tubes.	2a. Best done while still warm after autoclave.	

WATER MONITORING PROCEDURE: Coliform Test by the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Twenty-four Hour Procedures (Continued)	3. Empty sterilized cultures into sink.		
	4. Wash and dry culture tubes, fermentation vials, and tube caps.	4a. Meets original cleanliness requirements of glassware. 4b. Tubes and caps ready for re-use.	
6. Disinfection	1. Disinfect laboratory bench top; wipe dry.	1a. Sponge and disinfectant; paper toweling.	
D. Forty-eight Hour Procedure			
1. Equipment Maintenance	1. Check, record, and adjust incubator temperatures.		
	2. Add water to pan in incubator as necessary.		
2. Disinfection	1. Disinfect lab bench top; wipe dry.		
3. Reading and Recording of Results	1. Remove the rack of cultures from the incubator to lab bench.		
	2. Shake culture rack gently.		
	3. Examine each tube for gas production and record results on data sheet.	3a. LLSTB tubes will be recorded under the "48" on the LLSTB column and the BGLBB tubes under the "24" column. 3b. Any amount of gas is always considered to be a "positive" (+) result.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Forty-eight Hour Procedures		3c. Assume that our "test" never shows the following recordings:	

Amount Sample ml	Preservative LLSTB		Confirmed BGLBB	
	24 hr	48 hr	24 hr	43 hr
10	+		+	
	−	+		
	−	−		
	−	−		
	+		−	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Transfers	1. Discard all of the BGLBB tubes which have the positive (+) recordings.	1a. This will be a total of one tube (see data sheet recordings in D.3.3c)	
	2. Discard all LLSTB tubes which have the negative (-) recordings.	2a. This will be a total of 2 tubes (D.3.3c) 2b. LLSTB tubes which show NO GAS production within 48 hours are to be considered as not having contained coliform bacteria.	
	3. Re-incubate any BGLBB tubes which were negative and assemble for transfer any positive LLSTB tubes.	3a. There will be one tube of BGLBB which must be re-incubated for an additional 24 hours at 35° ± 0.5°C (D.3.3c). 3b. There will be one positive LLSTB tube (D.3.3c).	
	4. Label required tube of sterile BGLBB.	4a. This is done so that the re-incubated (24 hour old) BGLBB tube will not be confused with the newly inoculated BGLBB tube since both have to be incubated for 48 hours.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Forty-eight Hour Procedures (Continued)	5. Transfer the positive LLSTB tube to the sterile BGLBB tube.	5a. Use 3 mm loop whicn is flamed prior to entry into the LLSTB to avoid contamination. 5b. Use one loopful of transfer from the LLSTB to BGLBB.	
	6. After transfer place LLSTB tube in discard basket.		
5. Incubation	1. Incubate inoculated BGLBB tube. 1a. (Alternate) If no cultures for this test procedure remain to be incubated, proceed to "Interpretation of Test Results" and continue as directed.	1a. 35 ± 0.5°C for 24 hours.	
6. Processing Discarded Tubes of Media	1. Sterilize discarded media.		
	2. Remove all labels from culture tubes.		
	3. Empty sterilized cultures into sink.		
	4. Wash and dry culture tubes, fermentation vials, and tube caps.		
7. Disinfection	1. Disinfect laboratory bench top; wipe dry.		
E. Seventy-two Hour Procedures			
1. Equipment Maintenance	1. Check, record, and adjust incubator temperatures.		

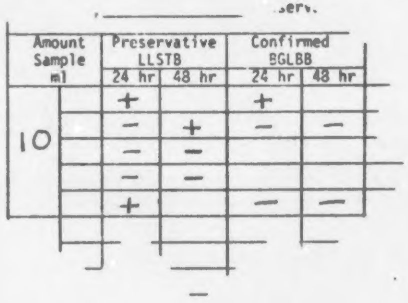
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Seventy-two Hour Procedure (Continued)	2. Add water to pan in incubator as necessary.		
2. Disinfection	1. Disinfect lab bench top; wipe dry.		
3. Reading and Recording of Results	1. Remove cultures from incubator to lab bench. 2. Shake cultures gently.		
	3. Examine each tube for gas production and record results on data sheet.	3a. In our continuing example, two tubes of BGLBB are to be examined--one of which will be a "48" entry and the other of the "24" column entry. 3b. Assume the following recordings will be made:	

Amount Sample ml		Preservative LLSTB		Confirmed BGLBB	
		24 hr	48 hr	24 hr	48 hr
10		+		+	
		−	+	−	
		−	−		
		−	−		
		+		−	−

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	4. Incubate any cultures which are still negative if they have not been incubated a full 48 hours.	4a. One BGLBB tube will have to be returned to the incubator since it is only 24 hours old and still negative. 4b. 35 ± 0.5°C for an additional and final 24 hours.	
4. Processing Discarded Tubes of Media	1. Sterilize discarded tubes of media. 2. Remove all labels from tubes.		

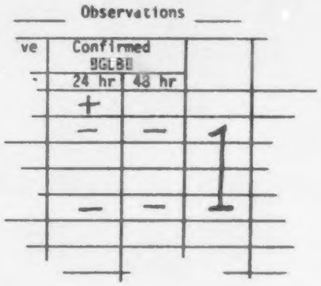
WATER MONITORING PROCEDURE: Coliform Test by the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Seventy-two Hour Procedures (Continued)	3. Empty sterilized tubes into sink.		
5. Disinfection	1. Disinfect lab bench top; wipe dry.		
F. Ninety-six Hour Procedures			
1. Equipment Maintenance	1. Check, record, and adjust incubator temperatures.		
	2. Add water to pan in incubator as necessary.		
2. Disinfection	1. Disinfect lab bench top; wipe dry.		
3. Reading and Recording of Results	1. Remove the BGLBB tube from incubator to lab bench.		
	2. Shake culture gently.		
	3. Examine tube for gas production and record results on data sheet.	3a. In our example assume the final recordings on the data sheet will be:	



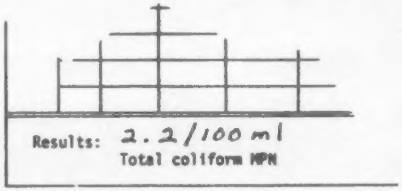
serv.

Amount Sample ml		Preservative LLSTB		Confirmed BGLBB	
		24 hr	48 hr	24 hr	48 hr
10		+		+	
		−	+	−	−
		−	−		
		−	−		
		+		−	−

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Ninety-six Hour Procedures (Continued)		3b. With final recordings completed, one can now proceed to "Interpretation of Test Results." 3c. Final entries could have been made as early as the 48-hour procedures up to these 96-hour procedures.	
4. Disinfection	1. Accomplish as in previous directives.		
5. Processing of Discarded Tubes	1. Accomplish as in previous directives.		
G. Interpretation of Test Results	1. Determine number of BGLBB tubes which are positive in the row of 5 tubes.	1a. NO consideration of presumptive test (LLSTB) for interpretation of test results. 1b. Our example (F.3.3) shows one positive BGLBB tube.	II.G.1
	2. Write the numbers in the data sheet.	2a.  2b. One of the five BGLBB tubes is positive.	
	3. Select from the proper table the MPN Index for the test result.		

Observations

ve	Confirmed BGLBB 24 hr	48 hr	
	+		
	–	–	1
	–	–	

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>G. Interpretation of Test Results (Continued)</td><td></td><td>3a. MPN Index for Various Combinations of Positive and Negative Results When Five 10-ml Portions Are Used
<table>
<tr><th>No. of Tubes Giving Positive Reaction out of 5 of 10 ml Each</th><th>MPN Index/ 100 ml</th></tr>
<tr><td>0</td><td><2.2</td></tr>
<tr><td>1</td><td>2.2 ←</td></tr>
<tr><td>2</td><td>5.1</td></tr>
<tr><td>3</td><td>9.2</td></tr>
<tr><td>4</td><td>16.</td></tr>
<tr><td>5</td><td>>16.</td></tr>
</table>
3b. For the example the location of the MPN index is 2.2/100 ml based on the single positive BGLBB result. The arrow locates the MPN Index.</td><td></td></tr>
<tr><td></td><td>4. Record the total coliforms per 100 ml on the laboratory data sheet.</td><td>4a. 
Results: 2.2/100 ml
Total coliform MPN</td><td></td></tr>
<tr><td>H. Reporting of Results</td><td>1. Report results as prescribed by State regulatory requirements.</td><td></td><td></td></tr>
</table>

WATER MONITORING PROCEDURE: Coliform Test by the MPN Method for Drinking Water

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II*	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analyses
VIII	Safety
IX	Records and Reports

*Training guide materials are presented here under the headings marked *. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.6.1	These MPN methods for determining bacterial numbers are based on the assumption that the bacteria can be separated from one another (by shaking or other means) resulting in a suspension of individual bacterial cells, uniformly distributed through the original sample when the primary inoculation is made. Test procedures are based on certain fundamental assumptions: a. First, even if only one living cell of the test organisms is present in the sample, it will be able to grow when introduced into the primary inoculation medium; b. Second, growth of the test organism in the culture medium will produce a result which indicates presence of the test organism; and c. Third, unwanted organisms will not grow, or if they do grow, they will not limit growth of the test organism; nor will they produce growth effects that will be confused with those of the bacterial group for which the test is designed.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
G.1	For purely qualitative aspects of testing for indicator organisms, it is convenient to consider the tests applied to one sample portion, inoculated into a tube of culture medium, and the follow-up examinations and tests on results of the original inoculation. Results of testing procedures are definite: positive (presence of the organism-group is demonstrated) or negative (presence of the organism-group is not demonstrated). The combination of positive and negative results is used in an application of probability mathematics to secure a single MPN value for the sample. To obtain MPN values, the following conditions must be met: a. The testing procedure must result in one or more tubes in which the test organism is demonstrated to be present; and b. The testing procedure must result in one or more tubes in which the test organism is not demonstrated to be present. The MPN value for a given sample is obtained through the use of MPN Tables. It is emphasized that the precision of an individual MPN value is not great when compared with most physical or chemical determinations.	

C.3.3 Interpretation of results on LLSTB:

Development of gas in this medium indicates that the lactose has been fermented. Fermentation of lactose with gas production is a basic characteristic of coliform bacteria. To meet the definition of coliforms, gas must be produced from lactose within 48 hours after being placed in the incubator. If a culture develops gas only after *more than 48 hours* incubation, then, by definition, it is *not a coliform*.

Meeting previously discussed assumptions (see I.B.6.1.1) usually makes it necessary to conduct the tests in a series of stages.

Features of a full, multi-stage test:

a. *First stage*: The culture medium usually serves primarily as an enrichment medium for the group tested. A good first-stage growth medium should support growth of *all* the living cells of the group tested, and it should include provision for indicating the presence of the test organism being studied. A first-stage medium may include some component which inhibits growth of extraneous bacteria, but this feature *never* should be included if it also inhibits growth of any cells of the group for which the test is designed. The Presumptive Test for the coliform group is a good example. The medium supports growth, presumably, of all living cells of the coliform group; the culture container has a fermentation vial for demonstration of gas production resulting from lactose fermentation by coliform bacteria, if present; and sodium lauryl sulfate may be included in one of the approved media for suppression of growth of certain non-coliform bacteria. This additive apparently has no adverse effect on growth of members of the coliform group in the concentrations used. If the result of the first-stage test is negative, the study of the culture is terminated, and the result is recorded as a negative test. No further study is made of negative tests. If the result of the first-stage test is positive, the culture may be subjected to further study to verify the findings of the first stage.

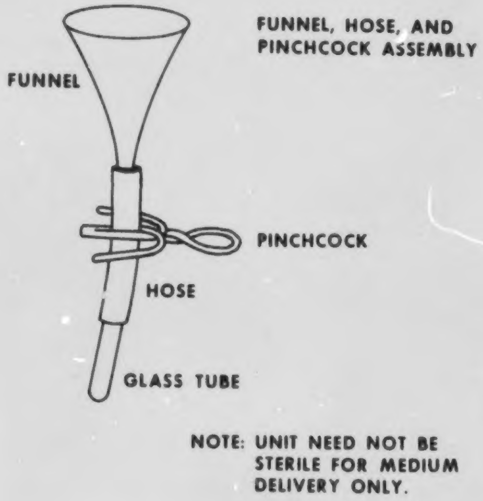
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1	Incubator must be of sufficient size for daily workload without causing crowding of tubes to be incubated. Considerations for choice of incubator type must relate to reliability of operation and not to cost or attractiveness of equipment.	
A.1.1	Incubator should be kept out of drafts or direct sunlight in order to prevent temperature inside the incubator from changing outside the temperature range specified (35° ± 0.5°C). Power supply should be selected so that there won't be too many pieces of equipment on the same circuit. Otherwise, circuits will be blown repeatedly.	Standard Methods for the Examination of Water and Wastewater, 14th ed. (1975) APHA, WPCF, AWWA, p. 880 (Hereafter referred to as: Std. Meth. 14: (page no.)
A.1.2	Mercury bulb thermometer usually used in most incubators. Recording thermometer is acceptable, but, it should be calibrated against a mercury bulb thermometer which has been certified by National Bureau of Standards. The NBS certified thermometer always should be used with its certificate and correction chart.	
A.1.3	Saturated relative humidity is required in order to make the incubation more efficient (heat is transferred to cultures faster than in a dry incubator). Furthermore, culture medium may evaporate too fast in a dry incubator.	
A.1.5	Allow enough time after each readjustment to permit the incubator to stabilize before making a new adjustment. At least one hour is suggested.	
A.1.6	Incubator temperature can be held to much closer adjustment if operated continuously. Temperature records should be kept in some form of permanent record. A temperature record book is suggested with daily recording of values. If a recording thermometer is used, the charts may be kept as permanent record; if so, be sure that the charts are properly labeled to identify the incubator and the period covered. Uniform temperature (35°C ± 0.5°C) is to be maintained on shelves in use.	
A.2.1-5	Since electric sterilizer will be operated intermittently, care should be taken that it is on a circuit which will not be overloaded when it is turned on.	Std. Meth. 14:881

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.2.1-5 (Continued)	A time and temperature record is maintained for each sterilization cycle. Temperature recordings can be retained for records.	
A.3.1	Autoclaves differ greatly in design and in method of operation. Some are almost like home-style pressure cookers; others are almost fully automatic. This is a subject which requires separate instruction; and should be related to the exact make and model of equipment you will use in your own laboratory. Vertical autoclaves and household pressure cookers may be used in emergency service if equipped with pressure gages and thermometers with bulbs positioned 1 inch above the water level. However, they are not to be considered the equivalent of the general purpose steam sterilizer recommended for permanent laboratory facilities. Their small size is inadequate for large-volume work loads, and they can be difficult to regulate. The following requirements must be met regarding autoclaves of sterilizing units: a. Reaches sterilization temperature (121°C), maintains 121°C during sterilization cycle, and requires no more than 45 minutes for a complete cycle. b. Pressure and temperature gages on exhaust side and an operating safety valve. c. No air bubbles produced in fermentation vials during depressurization. d. Record maintained on time and temperature for each sterilization cycle.	Std. Meth. 14:881
A.4.1-2	Distilled water in a bacteriological laboratory must not contain substances which will prevent any bacteria from growing in culture medium in which the distilled water is used or will be highly nutritive. There are procedures for testing quality of distilled water; but these should be undertaken only by professional bacteriologists or in laboratories where this is done regularly. Use only glass stills or block tin lined stills.	Std. Meth. 14:645-49 14:888-89 Training Manual (EPA Current Practices in Water Microbiology

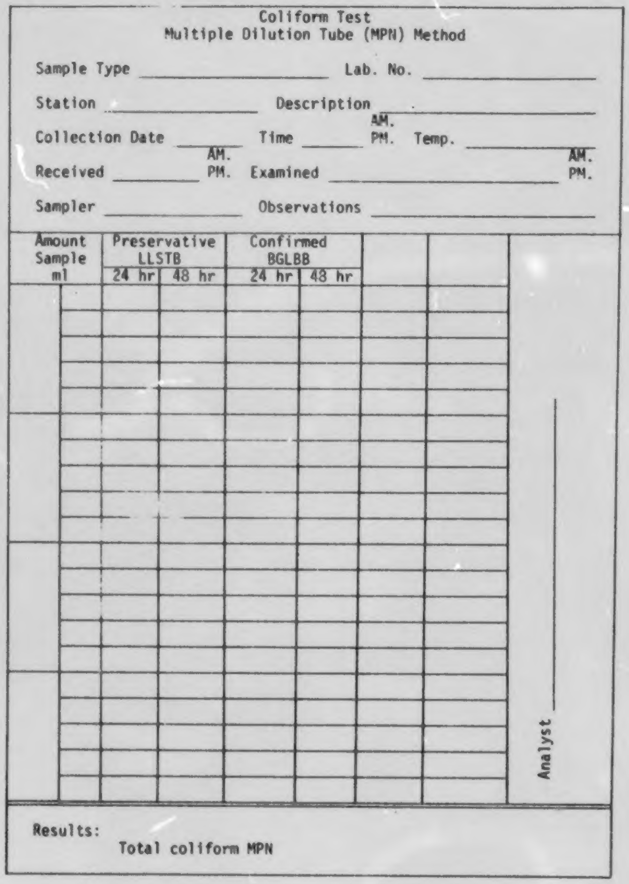
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.4.1-2 (Continued)	Requirements for distilled water include the following:	

Test	Analysis Requirement	Conducted
pH	4.5-8.5	Monthly
Conductivity	0.1 megohm as resistivity or <5.0 micromhos/cm at 25°C	Monthly
Trace metals:		
A single metal	Not greater than 0.05 mg/l	
Total metals	Equal to or less than 1.0 mg/l	Annually
Test for bactericidal properties of distilled water ("Standard Methods," 14th ed., p. 887)	0.8-3.0	Annually
Free Chlorine residual	0.0	Monthly
Standard plate count	Less than 10,000/ml	Monthly

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.5.1	pH Meter: See cited reference	Std. Meth. 14:882
A.6.1-4a	Glassware: See cited reference on pipets and graduated cylinders, media utensils, bottles.	Std. Meth. 14:882-885
A.6.1-4b	Glassware can be checked for bacteriostatic or inhibitory residues by a bacteriological test procedure which, like the distilled water suitability test, should be undertaken only by professional bacteriologists or in laboratories where this test is done on a regular basis.	
A.8.1-6	Sample bottles: Wide-mouthed glass-stoppered bottles suggested, but other styles acceptable. If glass-stoppered bottles are used, a strip of paper should be placed in the neck of the bottle before placing the stopper in place in preparation for sterilization. This prevents the glass stopper from "freezing" in place during sterilization. The paper strip is discarded at the time of sample collection.	Std. Meth. 14:884 14:904
A.9.1-6	Pipets: This procedure is described in terms of reusable glass pipets. However, single-service prepackaged glass or plastic pipets may be purchased and used, if preferred. In case of use of single-service pipets, they will be sterile when purchased, are used one time, and discarded immediately after use. Accordingly, in the step-by-step procedures disregard any instructions about preparation of pipets for reuse in case of using single-service pipets.	Std. Meth. 14:882-883

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.9.7	Passing the opened can of pipets through a flame burns off excess cotton wisps sticking out of the mouthpiece of the pipet. If this is not done, it is almost impossible to control sample measurement accurately. Some workers may elect to accomplish this step prior to the sterilization procedure.	
A.10.3b	 FUNNEL, HOSE, AND PINCHCOCK ASSEMBLY FUNNEL PINCHCOCK HOSE GLASS TUBE NOTE: UNIT NEED NOT BE STERILE FOR MEDIUM DELIVERY ONLY.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.3.1	There is no such thing as a "standard" data sheet for bacteriological tests. A simplified data sheet is shown below:	



Coliform Test
Multiple Dilution Tube (MPN) Method

Sample Type ________ Lab. No. ________

Station ________ Description ________

Collection Date ______ Time ______ AM. PM. Temp. ________

Received ________ AM. PM. Examined ________ AM. PM.

Sampler ________ Observations ________

Amount Sample ml		Preservative LLSTB		Confirmed BGLBB			
		24 hr	48 hr	24 hr	48 hr		

Analyst ________

Results:
Total coliform MPN

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	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.4.2	Transfers of LLSTB Transfers can be made, as indicated, with a wire loop having a diameter of at least 3 mm. An alternate method of transfer authorizes the use of an "applicator stick" which is a single service hardwood transfer device. Its dimensions are 0.2 to 0.3 cm in diameter and 2.5 cm longer than the test tube used in the analysis. The term single service denotes that the stick is pre-sterilized and used for a single transfer (LLSTB to BG) and then discarded in the pan containing disinfectant and a new sterile stick used for the next tube to be transferred. Use of this stick technique makes the gas burner unnecessary for the transfer process.	Std. Meth. 14:922

This outline was prepared by: Rocco Russomanno, Microbiologist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

COMPLETED TEST FOR THE MPN METHOD FOR DRINKING WATER

as applied in

DRINKING WATER TREATMENT FACILITIES
and in the
DISTRIBUTION SYSTEMS OF DRINKING WATER TREATMENT FACILITIES

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

BA.MET.lab.WMP.5a.10.78

Completed Test for the MPN Method for Drinking Water[1]

Completed Test

Applied to 10 percent of all positive samples each quarter
Applied to all positive confirmed tubes in each test completed
Positive confirmed tubes streaked on EMB plates for colony isolation
Plates adequately streaked to obtain discrete colonies
Incubated at 35° ± 0.5°C for 24 ± 2 hours
Typical nucleated colonies, with or without sheen, on EMB plates selected for completed test identification
If typical colonies absent, atypical colonies selected for completed test identification
If no colonies or only colorless colonies appear, confirmed test for that particular tube considered negative
An isolated typical colony or two atypical colonies transferred to lauryl tryptose broth
Incubated at 35° ± 0.5°C; checked for gas within 48 ± 3 hours

Cultures producing gas in lauryl tryptose broth within 48 ± 3 hours are considered coliforms.

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[1] MINIMUM REQUIREMENTS

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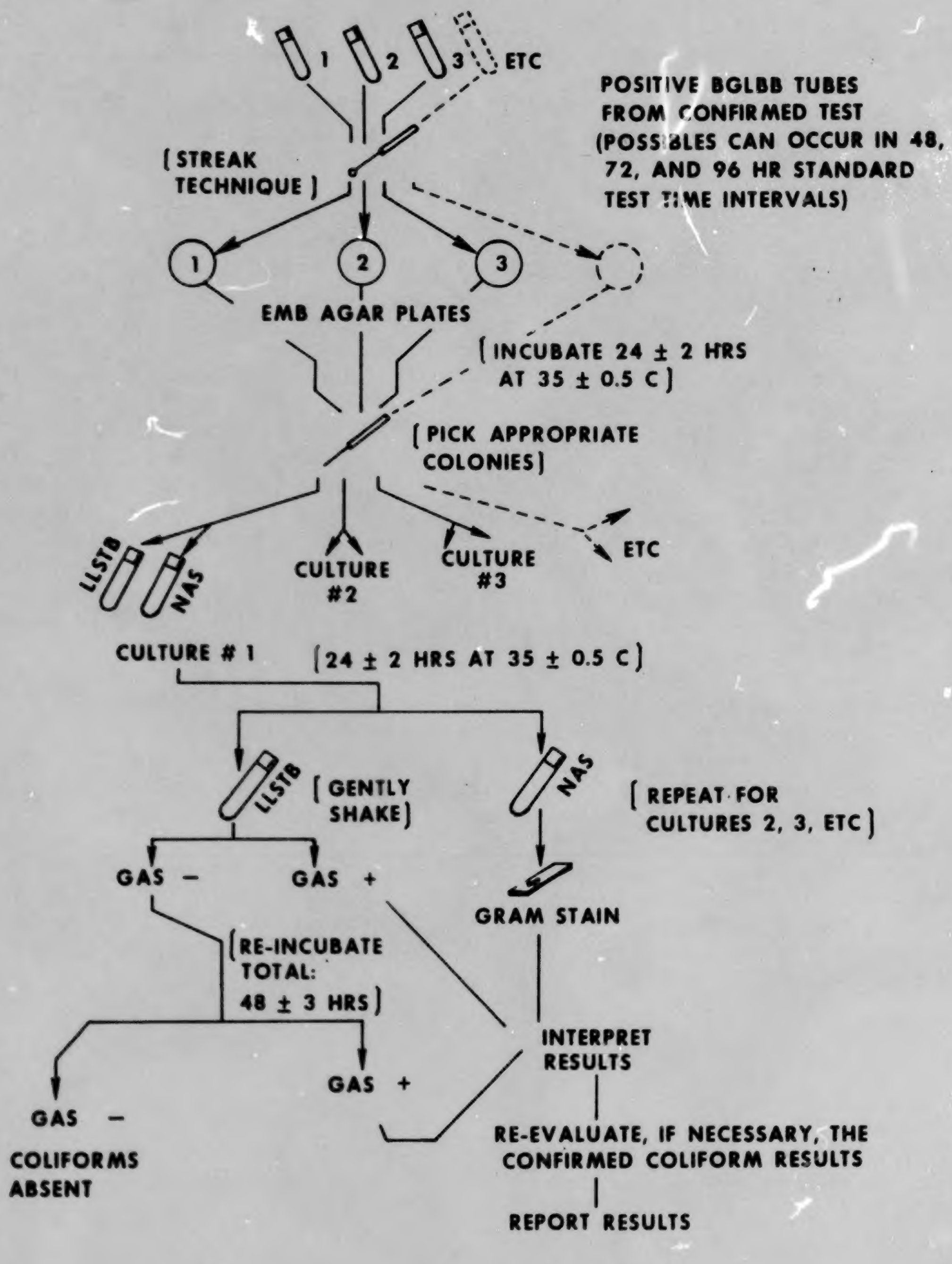
COMPLETED TEST SCHEMATIC


1
2
3
ETC
POSITIVE BGLBB TUBES FROM CONFIRMED TEST (POSSIBLES CAN OCCUR IN 48, 72, AND 96 HR STANDARD TEST TIME INTERVALS)
(STREAK TECHNIQUE)
1
2
3
EMB AGAR PLATES
(INCUBATE 24 ± 2 HRS AT 35 ± 0.5 C)
(PICK APPROPRIATE COLONIES)
LLSTB
NAS
CULTURE #2
CULTURE #3
ETC
CULTURE # 1
(24 ± 2 HRS AT 35 ± 0.5 C)
LLSTB
(GENTLY SHAKE)
NAS
(REPEAT FOR CULTURES 2, 3, ETC)
GAS −
GAS +
GRAM STAIN
(RE-INCUBATE TOTAL: 48 ± 3 HRS)
INTERPRET RESULTS
GAS +
GAS −
COLIFORMS ABSENT
RE-EVALUATE, IF NECESSARY, THE CONFIRMED COLIFORM RESULTS
REPORT RESULTS

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

1. Analysis Objectives:

In drinking water control testing, this test is part of the Standard Test for Coliforms and is one of the two tests of choice for reporting purposes. The completed test must be applied in the examination of drinking water to at least ten (10) percent of all positive samples in each quarter, and, when a specific sample is being tested, applied to all positive confirmed tubes of that sample.

Repeat samples from the same location that consistently show three or more positive 10 ml portions should be tested by this procedure.

2. Brief Description of Analysis:

All positive tubes of BGLBB (brilliant green lactose bile broth) from the confirmed test of the Standard Coliform Test are individually and aseptically transferred onto EMB Agar by the streaking technique. After incubation for 24 ± 2 hours at 35 ± 0.5°C, one or more typical isolated colonies are selected (dark-centered with or without sheen formation) or two or more atypical (if only these are present) isolated colonies (opaque; un-nucleated; mucoid; pink) from each plate and transferred to LLSTB (lactose lauryl sulfate tryptose broth) and a Nutrient Agar Slant (NAS). Thus, each selected pure culture is transferred to LLSTB and NAS and incubated for 24 ± 2 hours at 35 ± 0.5°C. Tubes are inspected at this time for gas formation in the LLSTB and growth on the NAS. A Gram Stain is prepared from the NAS at this time and the slant aseptically (handled with sterile technique) manipulated and preserved under refrigeration for possible future need. A positive (gaseous) LLSTB is data recorded and discarded while a negative (non-gaseous) tube is re-incubated for an additional 24 hours (total of 48 ± 3 hours) when it is again inspected for gas production.

Coliforms are considered to have populated the original BGLBB tubes if pure culture gram-negative, non-sporeforming rods, which gaseously fermented lactose were isolated by this procedure. Any other results are considered to be the actions of non-coliforms except in the case of lactose fermenters which are caused by mixed culture (two or more different organisms consisting of gram-positive and gram-negative forms). In this case, the retained Nutrient Agar Slant is restreaked on EMB and the subsequent procedures repeated to attempt to isolate the gram-negative pure culture having the coliform characteristics mentioned. Adjustments, if any, are made to the tube codings and the MPN re-calculated to give an MPN completed result which is now the required reportable result.

This procedure conforms to the Standard Total Coliform MPN Tests as described in Standard Methods for the Examination of Water and Wastewater, 14th ed. (1975). p. 914

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

Equipment and Supply Requirements

A. Capital Equipment

1. *Autoclave, providing uniform temperatures up to and including 121°C, equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperature within 30 minutes
2. Balance, 0.1 g sensitivity at load of 150 g
3. Air *incubator to operate at 35°C ± 0.5°C
4. Oven, *hot-air sterilizing or drying, to give uniform temperatures and with suitable thermometer to register accurately in range of 160-180°C
5. pH meter, accurate to at least 0.1 pH unit, with standard pH reference solution(s)
6. Water distillation apparatus, (glass or block tin), or source of distilled water suitable for bacteriological operations
7. Microscope, compound, oil immersion lens, Abbé condenser

B. Reusable Supplies:

1. Apron or coat suitable for laboratory
2. Baskets, wire, for discarded cultures
3. Hotplate with magnetic whirl feature, if desired
4. Burner, gas, Bunsen burner type
5. Counter, colony, Quebec type, Darkfield Model with guide plate
6. Inoculation loop and needle, 3 mm diameter for loop and both of nichrome or platinum-iridium wire, 26 B&S gauge, in holders
7. Pan, to receive discarded contaminated pipets and glassware (must contain disinfectant before use)
8. Racks, culture type*, 10 x 5 openings, to accept tubes at least 25 mm in diameter
9. Sponge, for cleaning desk top
10. Tubes, culture*, 150 x 18 mm (metal caps for fermentation and screw-cap for slants)
11. Tubes, fermentation*, 75 x 10 mm vials to be inverted in culture tubes
12. Flasks, Erlenmeyer: 500 ml; 300 ml; 250 ml
13. Graduates: 500 ml; 250 ml

Equipment and Supply Requirements (Continued)

C. Consumable Supplies:

1. Bibulous paper
2. Dishes, petri, 100 x 15 mm, sterile plastic, disposable
3. Disinfectant, for bench tops (Can use household bleach solution prepared according to instructions on bottle)
4. Distilled water, suitable for bacteriological cultures (note distillation apparatus required in capital equipment)
5. Eosin methylene blue agar, dehydrated medium (Levine modification)
6. Gram stain solutions, complete set
7. Lactose Lauryl Sulfate Tryptose Broth, dehydrated medium
8. Nutrient Agar, dehydrated medium
9. Slides, microscopic, glass, 1" x 3"
10. Foil, aluminum
11. Matches
12. Wax pencils (recommend soft as equivalent to Blaisdell 169T)

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*Items marked are needed in quantities or require size or space allowances which cannot be specified here, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of "Standard Methods for the Examination of Water and Wastewater."

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures		Aa. All pre-test procedures completed before starting other first-day procedures.	
1. 35°C Incubator Set-Up, Adjustment	1. Place 35°C incubator in permanent location.	1a. Out of drafts or places where it will be in sunlight part of day. 1b. Location convenient to laboratory bench 1c. Convenient source of electric power.	V.A.1 V.A.1.1 (p. 46)
	2. Install thermometer.	2a. Thermometer functions at least in 30°-40°C range and have intervals of 0.5° or less indicated. Meets NBS standards. 2b. Location should be central in incubator. 2c. Mercury bulb thermometer should be fitted with cork or rubber stopper and mounted in small bottle filled with liquid (glycerine, water, or mineral oil).	V.A.1.2 (p. 46)
	3. Install shallow pan of water in bottom of incubator.	3a. In most incubators a pan having about 1 square foot of area, with water about 1 inch deep, is satisfactory. 3b. Maintains condition of saturated relative humidity, required in bacteriological incubator. 3c. Requires daily check, with addition of water as necessary, to keep water in pan at all times.	V.A.1.3 (p. 46)
	4. Connect incubator to electric power source.	4a. Many incubators have pilot light to indicate power turned on.	
	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for method temperature adjustment. 5b. Operation must be at 35° ± 0.5°C. 5c. Allow about 1 hour between adjustments.	V.A.1.5 (p. 46)
	6. Operate bacteriological incubator continuously.	6a. Requires daily check with written temperature record, with adjustment and water addition as necessary.	V.A.1.6 (p. 46)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued) 2. Oven, Sterilizer-Drier, Setup	1. Place oven sterilizer in permanent location.	1a. Convenient to source of electric power usually on table or bench.	V.A.2.1-5 (p. 47)
	2. Install thermometer.	2a. Should indicate the 160° - 180°C range, be accurate within this interval, and be marked in 1.0 degree intervals.	
	3. Connect oven sterilizer to power source and turn on.	3a. Usually has pilot light to indicate power on.	
	4. Adjust temperature to stabilize at required temperature.	4a. Operated as near to 170°C as possible; not lower than 160° or higher than 180°C.	
	5. Operate oven sterilizer only when needed. Turn off when not in use.	5a. Turned ON in advance of need to permit reaching required temperature before introducing material. 5b. Oven used to sterilize or dry glassware, metal objects. 5c. Oven sterilizer not used with culture media, solutions, plastics, rubber objects, or with anything containing or including these. 5d. Paper-wrapped glass pipets, graduates, flasks, etc. may be sterilized in oven sterilizer.	
3. Autoclave Setup	1. Install and operate autoclave according to manufacturer's instructions.	1a. Autoclaves extremely variable in design and operation; also, potentially dangerous. 1b. Used to sterilize objects made of, or including liquids, rubber, culture media. 1c. Glassware may be autoclave sterilized but must be dried afterward. 1d. Most plastics not sterilized in autoclave; plastics usually require chemical sterilizers. 1e. Autoclave usually operated at 121°C for 15 min. 1f. Sterilized media must be removed from autoclave as soon as possible after autoclave is reopened.	V.A.3.1 (p. 47)

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)			
4. Water Distillation Equipment	1. Install and operate in accordance with manufacturer's instructions.	1a. Must produce distilled water meeting quality requirements for bacteriological tests.	V.A.4.1-2 (p. 47)
	2. Operate as required to maintain adequate supplies of distilled water.	2a. Reserve supplies kept in borosilicate glass carboys or in plastic carboys made of material which will not dissolve substances which will affect growth of bacteria. 2b. Same distillation apparatus used for bacteriological purposes may be used for chemical reagents.	
5. pH meter	1. Have unit available and operable.	1a. Unit for pH check on finished culture media.	V.A.5.1 (p. 48)
6. Glassware	1. Wash all glassware in hot detergent solution.	1a. Nontoxic detergent. 1b. Be sure all contents and markings are washed away.	V.A.6.1-4a (p. 48)
	2. Rinse at least once in hot tap water.		
	3. Rinse in distilled water, at least 6 successive times, and		
	4. Dry in air or oven.	4a. No visible spots or scum; glass should be clean and sparkling. 4b. Glassware suitable for use in bacteriological operations.	V.A.6.4b (p. 48)

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued) 7. Preparation of Lactose Lauryl Sulfate Tryptose Fermentation Broth (LLSTB)	1. Weigh 8.9 grams of dehydrated Lactose Lauryl Sulfate Tryptose Broth. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media takes moisture out of air; can become caked. 1b. Caked media unsatisfactory; should be discarded.	
	2. Dissolve in 250 ml distilled water.	2a. Use a 500 ml Erlenmeyer flask. 2b. Gentle heat (no boiling) if necessary to complete dissolving medium. Usually a vigorous agitation will completely dissolve the medium.	
	3. Place 10.5 ml of the solution of prepared LLSTB in each culture tube.	3a. Use 150 x 18 mm tubes. 3b. A 25 ml pipet, automatic pipetter, or funnel, hose and pinchcock assembly are acceptable. 3c. Accuracy of delivery: ± 0.5 ml. 3d. Approximately 23 tubes will be necessary.	V.A.7.3 (p. 48)
	4. Insert one fermentation vial into each tube of medium, open end down.	4a. Tubes and vials washed as indicated previously. 4b. Use 75 x 10 mm tubes.	
	5. Place tube cap on each tube of culture medium.	5a. After all tubes have been filled and have individual vial.	
	6. Sterilize in autoclave.	6a. Within 1 hour after medium prepared. 6b. Sterilization at 121°C for 15 minutes. 6c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" mode of steam removal.	
	7. Cool medium to room temperature.	7a. Medium ready for use when cool and individual vials are completely filled with fluid. No bubbles must be present. Wait for complete cooling before checking for bubbles.	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	8. Check pH of finished medium.	8a. Should be pH 6.7 - 6.9. It is rare that deviations occur with this preparation.	
	9. If final pH is not satisfactory, discard medium and prepare new batch with pH adjustment before sterilization.	9a. pH value ordinarily drops about 0.2 pH unit. 9b. Check for dirty glassware, acid residues in glassware, etc.	
	10. Store medium in cool dark place.	10a. Not in refrigerator. Usually in laboratory cabinet in darkness. 10b. May be stored up to 1 week if evaporation not more than 10% in loose-fitting capped tubes. With screw-capped tubes, medium should be held no longer than 3 months.	
8. Preparation of Eosin Methylene Blue Agar (EMB Agar)	1. Weigh 7.5 grams of dehydrated eosin methylene blue agar. Close cover of bottle of dehydrated medium tightly after removal.	1a. Use only Levine's modification as this medium has a number of modifications for differing purposes. 1b. Dehydrated media takes moisture out of air; can become unacceptably caked.	
	2. Dissolve in 200 ml distilled water.	2a. Use a 300 ml Erlenmeyer flask with double layer foil cap. 2b. Heat to boiling to dissolve completely. Do not prolong boiling. 2c. Frequent agitation is necessary to prevent burning of medium. 2d. All of the agar must be in solution. Agar will be recognized as particulate matter along the sides of the flask. Gently swirl flask until all of this material is off of sides and into medium.	V.A.8.2c (p. 48)

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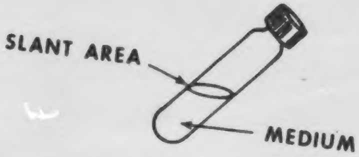
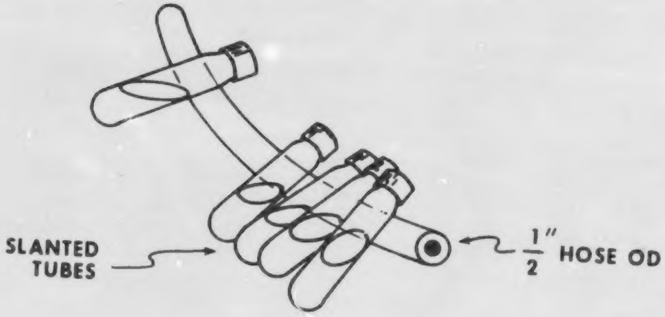
WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	3. Sterilize medium in autoclave.	3a. For 15/15 to effect complete sterilization (15 psi for 15 minutes). 3b. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" mode of steam removal.	
	4. Cool medium to 50-60°C and pour into sterile petri dishes.	4a. Can also be poured "hot" from autoclave with precautions, such as using asbestos glove, for personal protection. 4b. A flocculant may form after autoclaving. Swirl flask gently during plate (dish) filling. 4c. About 10-12 mls/plate. About 15 plates will be required. 4d. Cover plates as they are poured. Do not place covers on bench where they can become contaminated.	
	5. Allow dishes to cool to room temperature and then dry.	5a. Agar will solidify and allow plate to be moved without disturbing medium. 5b. Invert plates (turn upside down) and place in 35° incubator overnight. This will allow plates to dry and remove excess moisture. 5c. Plates can be used when agar surface is "dry" (does not have water droplets).	
	6. Check pH of one of the plates.	6a. Insert pH meter probes into agar medium using one of the plates of the batch. 6b. Should read 7.0 - 7.2. 6c. Discard plate after measuring pH. Alternately, to save medium, one could fill a small clean receptacle, or, a 60 x 15 mm petri dish for this check. 6d. Out of range reading denotes unacceptable procedure, equipment, or materials used (dirty glassware, poor water supply, overheating, etc.). Discard plates and rectify problem.	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	7. Label and date batch of plates. Store either at room temperature when use is made of plates within several days or in sealed plastic bags, at 4°C.	7a. Can be kept for one month under refrigeration as described. Plates may have to be re-dried in the incubator overnight (inverted) after removal from refrigerator.	
9. Prepare Gram-Stain Solutions	1. Prepare solutions as recommended by manufacturer.	1a. Premixed dyes will probably only require dilution. 1b. If desirous to prepare dyes from scratch, consult Standard Methods for procedure.	Std. Meth. 14:918-919
10. Prepare Nutrient Agar Slants (NAS)	1. Weigh 2.9 grams of dehydrated nutrient agar. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media takes moisture out of air; can become caked. 1b. Caked media unsatisfactory; should be discarded.	
	2. Dissolve in 125 ml distilled water.	2a. Use a 250 ml Erlenmeyer flask with double layer foil cap. 2b. Heat to boiling to completely dissolve. 2c. Frequent agitation is necessary to prevent burning of medium. 2d. All of agar must be in solution. Agar will be recognized as particulate matter along the sides of the flask. Gently swirl flask until all of this material is off of sides and into medium.	V.A.8.2c (p. 48)
	3. Dispense 6-7 mls of medium into screw-cap tubes.	3a. Use 150 x 18 mm screw-cap tubes. 3b. A 10 ml pipet, automatic pipetter, or funnel, hose, and pinchcock assembly are acceptable. 3c. Approximately 25 tubes will be required.	V.A.7.3 (p. 48)
	4. Place screw caps loosely on each tube which are packed loosely in a test-tube rack, beaker, etc.	4a. Allows steam to penetrate to medium.	

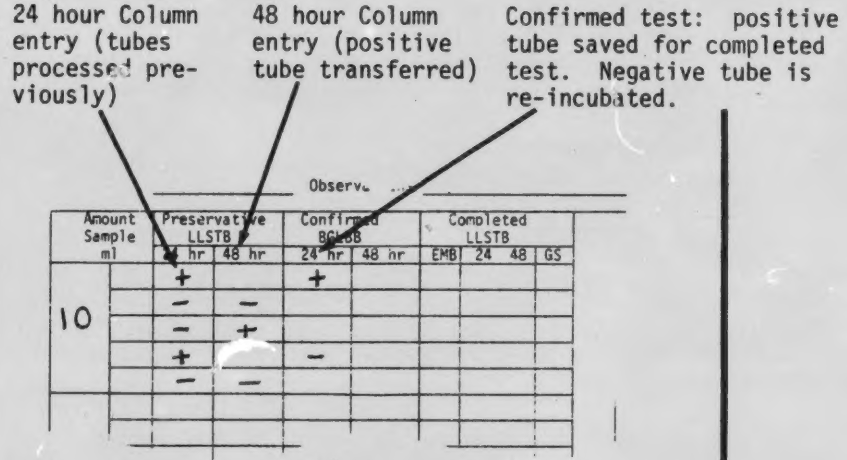
WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	5. Sterilize tubes in autoclave.	5a. For 15/15 to effect complete sterilization (15 psi for 15 minutes). Remove medium as soon as possible after cycle (slow vent mode) is completed.	V.A.3.1 (p. 47)
	6. Tighten caps and slant hot medium.	6a. Tight caps will prevent further loosening and possible contamination. 6b. Necessary to slant while hot so that medium will not solidify in upright position. 6c. "Slanting" is done to allow a large surface area for growth of bacteria.  6d. Apparatus for tube holding while in the slanted position can range from expensive "angle" controlled supports to as simple and effective a method as below:  	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	7. Allow tubes to solidify before removing from slanted position and placing in test tube rack. 8. Date and label medium as nutrient agar. Store in refrigerator.	7a. Solidified tubes can be picked up and will retain "slanted" position of medium. Tubes will start to harden below 40°C and take on an "opaque" form as they harden. 8a. Temperature 1-4.4°C. 8b. Can be stored for up to 3 months (if kept in dark and evaporation is not excessive (less than .25 ml).	
11. Final Equipment and Supply Check	1. Check to be sure that all equipment and supplies, solutions, and prepared media are ready before starting sample examination.	1a. Check general list of equipment and supplies. 1b. Each test requires: 1 - 5 EMB agar plates 1 - 10 Nutrient agar slants 1 - 10 LLSTB tubes Bacteriological loop Bacteriological needle 1 - 10 Microbiological slides Gram stain reagents, set Since, as shown, the numbers of items can vary (depending upon the number of confirmed test positives and subsequent EMB colony forms) this WMP (Water Monitoring Procedure) will specifically pick a hypothetical situation which will give the reader a cross-section of conditions which could occur.	
B. Initial Procedures 1. Equipment Maintenance	1. Check, record, and adjust incubator temperature.	1a. See A.1.1.1-6. 1b. Should be in operating condition since MPN test's earlier phases are in progress (Presumptive and Confirmed tests).	
	2. Add water to pan in incubator as necessary.		

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued)			
2. Data Sheet Inspection	1. Locate data sheet and verify that the required sample is being processed.	1a. A "new" data sheet does not have to be initiated since the sample is already being processed.	VII.B.2 (Suggested Data Sheet) (p. 50)
	2. Use active sheet of the 48 hour MPN test (partial completion of the confirmed test) with 48 hour presumptive tubes and 24 hour confirmed tubes "saved."	2a. A typical sheet may look like this: (Test Portion)	



24 hour Column entry (tubes processed previously)

48 hour Column entry (positive tube transferred)

Confirmed test: positive tube saved for completed test. Negative tube is re-incubated.

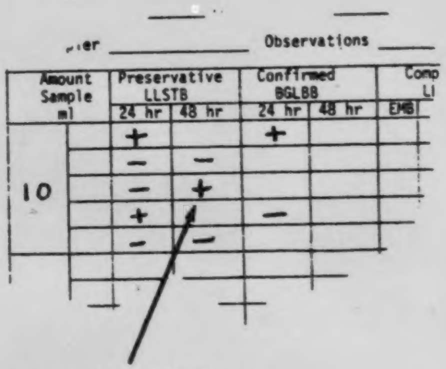
Observe

Amount Sample ml		Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24	Completed LLSTB 48	Completed LLSTB GS
10		+		+					
		−	−						
		−	+						
		+		−					
		−	−						

2b. Note that 10 ml volumes were used for each of the 5 tubes for the presumptive test.

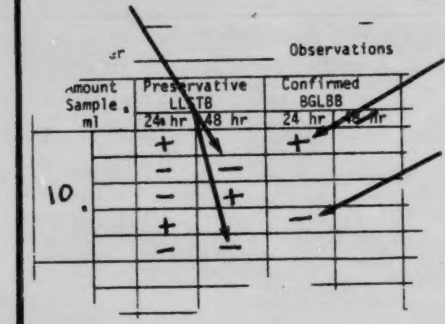
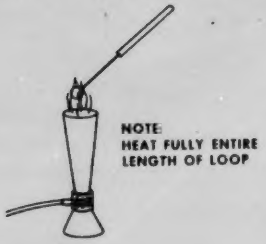
2c. Tubes "saved" will be used to initiate the completed test.

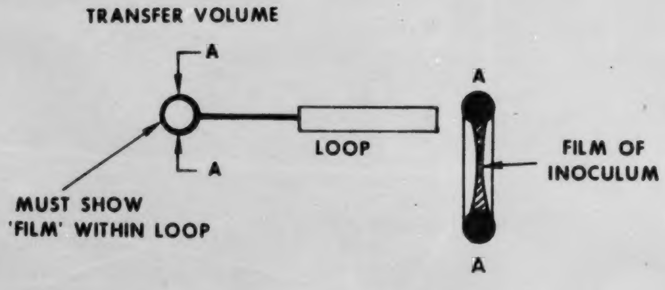
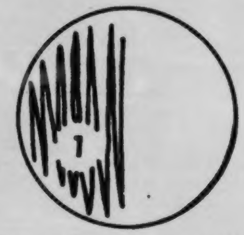
WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

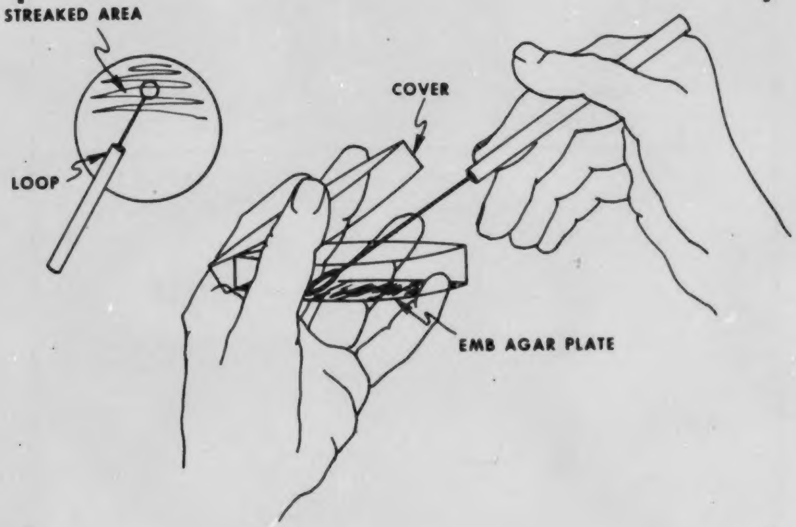
<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>B. Initial Procedures (Continued)</td><td></td><td></td><td></td></tr>
<tr><td>3. Lab Bench Disinfection</td><td>1. Disinfect laboratory bench; wipe dry.</td><td>1a. Sponge and disinfectant; paper toweling.</td><td></td></tr>
<tr><td>4. Continue Standard MPN Test Procedure</td><td>1. Transfer positive LLSTB tubes of the presumptive stage.</td><td>1a. From data sheet (B.2.2.2a), note that one tube will be transferred from the presumptive stage to the confirmed stage:

er Observations
<table>
<tr><th>Amount Sample ml</th><th></th><th>Preservative LLSTB 24 hr</th><th>48 hr</th><th>Confirmed BGLBB 24 hr</th><th>48 hr</th><th>Comp Ll EMB</th></tr>
<tr><td>10</td><td></td><td>+</td><td></td><td>+</td><td></td><td></td></tr>
<tr><td></td><td></td><td>−</td><td>−</td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td>−</td><td>+</td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td>+</td><td></td><td>−</td><td></td><td></td></tr>
<tr><td></td><td></td><td>−</td><td>−</td><td></td><td></td><td></td></tr>
</table>
Transfer this to BGLBB

1b. Progress of this transfer will be monitored for possible inclusion to the completed test.</td><td>Std. Meth. 14:917</td></tr>
</table>

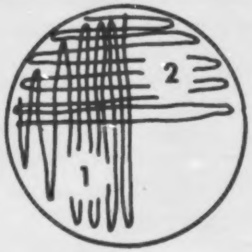
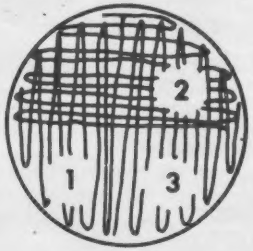
WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued) Completed Test Start: First Day Procedure 5. Select BGLBB Positives from Confirmed Test	1. Select "positives" from confirmed "24" hour tubes for processing.	1a. One positive BGLBB tube is to be processed: Disregard these negatives in coliforms absent (see schematic).  Observations Amount Sample ml Preservative LLSTB 24 hr 48 hr Confirmed BGLBB 24 hr 48 hr 10. + − − + − − + − + − This is the tube to be processed to EMB. Negative tube to be re-incubated as per Confirmed test requirements (Std. Meth. 14:920).	
6. Prepare EMB Agar Plates	1. Shake positive BGLBB tube vigorously. 2. Sterilize a bacteriological loop.	1a. Allows organisms to be suspended in the broth. 2a. Heat in burner to redness all the way to handle:  NOTE: HEAT FULLY ENTIRE LENGTH OF LOOP	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued)	3. Allow loop to cool (5-10 seconds).	3a. Avoids possible spattering when loop is inserted into tube.	
	4. Remove cap from BGLBB tube.		
	5. Insert loop into broth to obtain film transfer. Cover tube and discard.	5a. "Film" within loop represents transfer volume.  TRANSFER VOLUME A A LOOP MUST SHOW 'FILM' WITHIN LOOP A FILM OF INOCULUM A	V.B.6.5 (p. 48)
	6. Streak transfer inoculation from loop to corner of EMB agar plate.	6a. Agar surface must be dry for satisfactory results. 6b. Streak the inoculation lightly back and forth over half the agar surface, as in ①, avoiding scratching or breaking the agar surface.  (Over for pictorial representation)	VII.B.6.6 (p. 50)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued)			
		6c. Use asceptic (sterile) technique to prevent contamination of medium. Close cover of petri dish when not streaking.	
	7. Sterilize (flame) loop and air-cool as before.		
	8. Streak another segment of plate to carry portion of inoculation into another area of medium.	8a. Turn petri dish about one-quarter-turn in the holding hand (allows easier streaking). 8b. Streak the loop's tip lightly back and forth over one-half the agar surface working from area ① into one-half the unstreaked area of the agar. (Over for pictorial representation)	

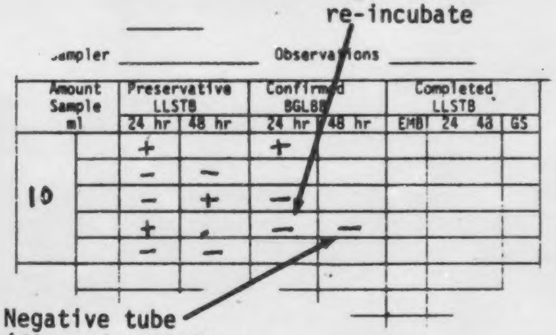
WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued)		 8c. Technique allows "dilution" of original heavy inoculum to occur into an area where less growth will now result.	
	9. Sterilize loop and air-cool.		
	10. Streak the remaining unstreaked area of medium.	10a. Turn the petri dish one-quarter-turn in the holding hand. 10b. Streak the tip lightly back and forth over one-half the agar surface, working from area ② into area ③.  10c. Do not allow any of streaks to touch original streaking area (separate ③ from ①). 10d. Further "dilution" will now occur to allow "pure" cultures to grow into colonies.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued)		10e. Close the culture container, and, until the colonies (bacterial growth forms) are picked, keep the top and bottom as a unit without allowing separation to occur.	
	11. Flame sterilize the loop and set it aside.		
	12. Invert dish (turn up-side-down) and identify.	12a. Use grease pencil (wax pencil) to label bottom of dish. 12b. A suggested labeling could be: 312 ←—Lab assigned no. for this sample 1 ←—Indicates 1st tube in row of 5 tubes	
	13. Incubate EMB agar plate.	13a. At 35° ± 0.5°C for 24 hours. 13b. Keep in inverted position (avoids water droplets, if formed, from falling on the medium surface and ruining the plate).	
C. Second-Day Procedures 1. Equipment Maintenance	1. Check, record, and adjust incubator temperature.		
2. Lab Bench Disinfection	1. Disinfect laboratory bench, wipe dry.	1a. Sponge and disinfectant; paper toweling.	
3. Data Sheet Recordings	1. Locate required data sheet.	1a. Sample "312" in our example.	
	2. Remove cultures from incubator and assemble with data sheet.	2a. 1 EMB plate (24 hours old) 1 BGLBB tube (24 hours old) 1 BGLBB tube (48 ± 3 hours old)	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedures (Continued)	3. Read BGLBB tubes for gas and record results.	3a. Any amount of gas is considered positive. Shake tubes gently before reading. 3b. Assume the following results:	(See schematic diagram) (p. 3)



Negative tube re-incubate

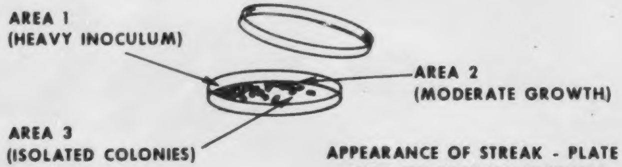
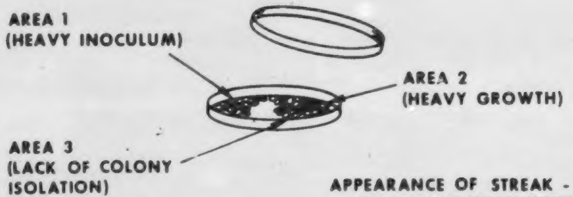
Sampler ______________ Observations ________

Amount Sample ml		Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24	Completed LLSTB 48	GS
10		+		+					
		−	−						
		−	+	−					
		+		−	−				
		−	−						

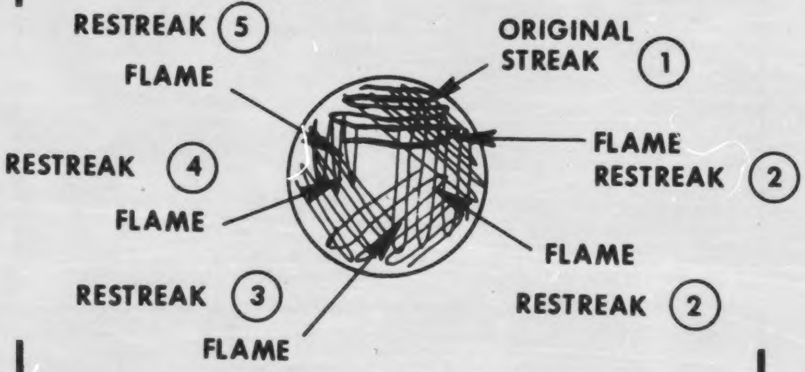
Negative tube (discard: coliforms absent)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	4. Discard any BGLBB tube which is negative in 48 hours.		
	5. Save any 24 hour BGLBB tube which is positive or negative.	5a. None are positive...this possibility would have made it necessary to streak an EMB agar plate. 5b. There is a negative...reincubate this for an additional 24 hours.	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

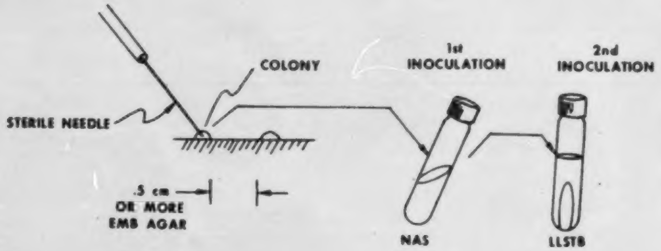
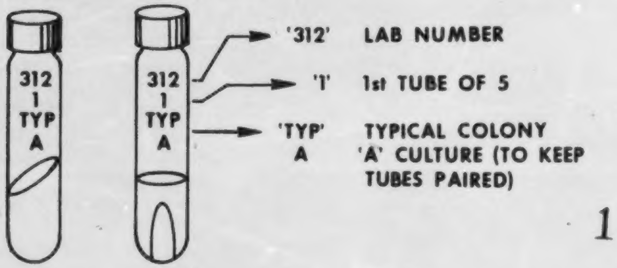
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedures (Continued) 4. EMB Agar Plate Inspection	1. Remove cover from EMB agar plate and inspect growth.	1a. Usual plate growth (colonies) will be as indicated:  AREA 1 (HEAVY INOCULUM) AREA 2 (MODERATE GROWTH) AREA 3 (ISOLATED COLONIES) APPEARANCE OF STREAK - PLATE AFTER INCUBATION INTERVAL OR OCCASIONALLY,  AREA 1 (HEAVY INOCULUM) AREA 2 (HEAVY GROWTH) AREA 3 (LACK OF COLONY ISOLATION) APPEARANCE OF STREAK - PLATE AFTER INCUBATION INTERVAL	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedures (Continued)		1b. In the case of isolated colonies, one could proceed to the next step of the completed test (C.5).	III.C.4.1b (p. 45)
		1c. In the case of a lack of isolated colonies, one must proceed to re-streak another plate to attempt isolation of a colony. As follows:	
		Reisolation Procedure	
		A. Flame sterilize a loop and air-cool.	
		B. Immerse the loop into an area which shows a representative growth mass. Occassionally, the loop must be touched to two or three masses to obtain this material.	
		C. Close cover and discard EMB plate.	
		D. Streak plate of fresh, dry EMB agar using the same techniques as previously outlined, except that it would be wise to allow more streaking sequences with an increased number of loop flamings. This would more likely ensure better isolation:	



E. Incubate as previously outlined.

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedures (Continued)		1d. Recommended technique is to pick pure colony and with a single transference, inoculate both the LLSTB and the NAS in a single motion.  COLONY 1st INOCULATION 2nd INOCULATION STERILE NEEDLE .5 cm OR MORE EMB AGAR NAS LLSTB NAS... Flame top of tube for about two seconds prior to entering with sterile (flamed) needle. Gently swab surface of agar medium. Replace screw-cap which is held in hand without contaminating during procedure. EMB... Discard plate after inoculation completed. LLSTB... Transfer inoculation directly to LLSTB tube. Return to colony is not necessary. Flaming of tube top not necessary. Shake needle in broth for transfer. 1e. Label tubes for identification. Such a labeling could be as follows:  312 1 TYP A 312 1 TYP A '312' LAB NUMBER '1' 1st TUBE OF 5 'TYP' TYPICAL COLONY A 'A' CULTURE (TO KEEP TUBES PAIRED)	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedures (Continued)		1f. Incubate tubes at 35° ± 0.5°C. 1g. Indicate the type of EMB plate colonies observed:	

Indicates typical colonies

Observations

Amount Sample ml		Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24	Completed LLSTB 48	Completed LLSTB GS
10		+		+		TYP			
		−	−						
		−	+	−					
		+		−	−				
		−	−						

1h. Indicate the numbers of pure cultures picked:

Indicates first positive culture "A"

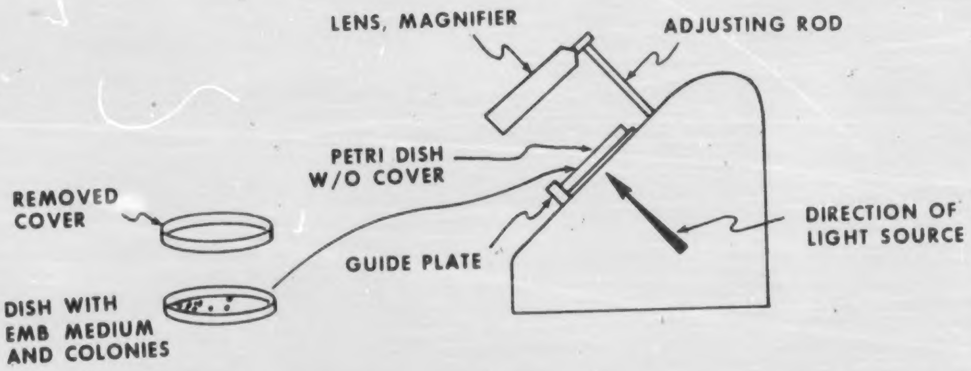
Observations

Amount Sample ml		Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24	Completed LLSTB 48	Completed LLSTB GS	
10		+		+		TYP				1A
		−	−							
		−	+	−						
		+		−	−					
		−	−							
										1B
										1C

If more than one pure culture was picked from the EMB plate derived from the first tube, indicate here as B, C, etc.

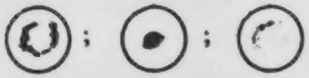
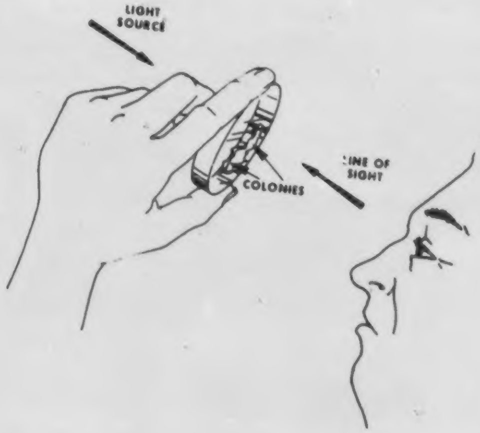
WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedures (Continued)		II. Atypical Colonies (Usually a Non-Coliform) These colonies may be opaque, unnucleated, mucoid, or pink after the prescribed incubation period.	
5. EMB Agar Plate Colony Transfer	1. Transfer pure cultures to LLSTB and NAS.	1a. Use flamed and air-cooled needle for fishing (picking). 1b. Use of colony counter as a magnification aid is recommended	



1c. Pick one or more typical colonies, or, two or more atypical colonies and transfer each of them into their own set of tubes (LLSTB and NAS).

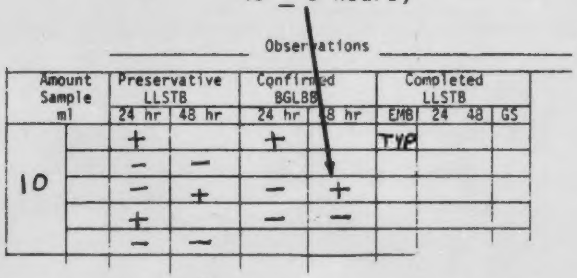
WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedures (Continued)		1d. Pure growths (colonies) can be regarded as falling into two groupings: I. Typical Colonies (Characteristic of Coliforms) Colonies with dark centers commonly termed "nucleated" or "fisheye" when viewed from the bottom of the plate:  ; etc. These colonies may or may not have a metallic-like sheen characteristic on the surface of the colony. 	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedures 1. Equipment Maintenance	1. Check, record, and adjust incubator temperature.		
2. Lab Bench Description	1. Disinfect laboratory bench	1a. Sponge and disinfectant; paper toweling.	
3. Data Sheet Recordings	1. Locate required data sheet.	1a. Sample "312" is our example.	
	2. Remove cultures from incubator and assemble with data sheet.	2a. 1 NAS, 1 LLSTB } Since we transferred one colony... could have been more cultures (i.e., at least 2 pairs if atypicals were present only). 24 hour incubation. 1 BGLBB 48 ± 3 hours of incubation	See C.3.3b
	3. Read BGLBB tube for gas and record results. Tube labeled: 312 3	3a. Any amount of gas is considered positive. Shake the tube gently before reading. 3b. Assume the following result:	

Positive tube (within 48 ± 3 hours)



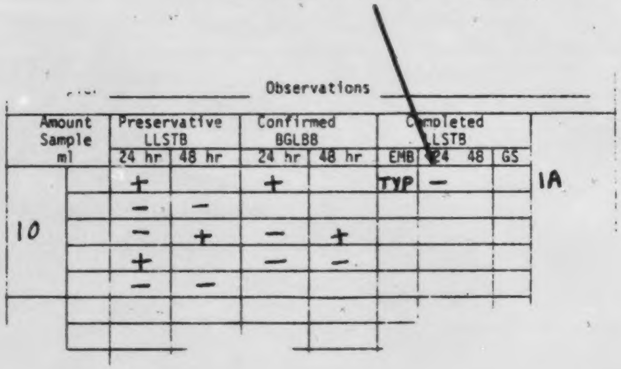
Observations

Amount Sample ml		Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24 48	Completed LLSTB GS
10		+		+		TYP		
		−	−					
		−	+	−	+			
		+		−	−			
		−	−					

3c. Retain the tube for further processing

(EMB → NAS, LLSTB)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedures (Continued)	4. Check NAS and LLSTB for growth and gas, respectively, and record results.	4a. Growth on the NAS is readily visible as an opaque mass which was not present on the sterile medium. No recordings are necessary for this growth--it will be used for a gram stain. 4b. Any amount of gas production in the LLSTB is considered positive. Shake the tube gently before reading. 4c. Assume the following result: *Negative tube (re-incubate for an additional 24 hours)	

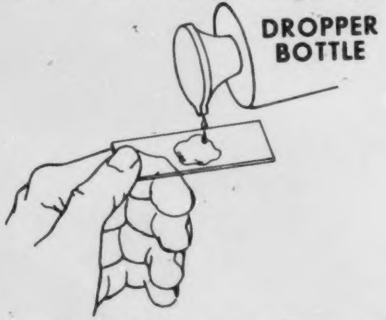


Amount Sample ml		Preservative LLSTB		Confirmed BGLBB		Completed LLSTB				
		24 hr	48 hr	24 hr	48 hr	EMB	24	48	GS	
10		+		+		TYP	−			1A
		−	−							
		−	+	−	+					
		+		−	−					
		−	−							

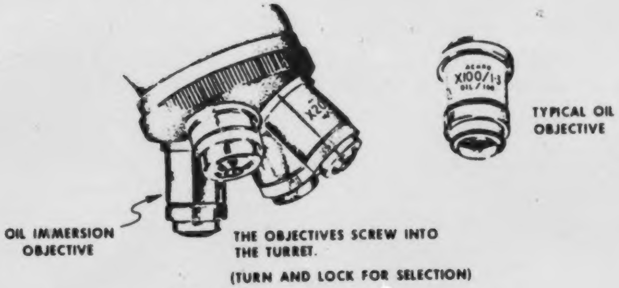
*Had this tube been positive, the completed test may have been terminated this third day instead of tomorrow (fourth day).

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Gram Stain Preparation	1. Assemble gram staining materials and culture.	1a. 1 Bacteriological glass slide 1 Dropper bottle containing ammonium oxalate-crystal violet dye 1 Dropper bottle containing Lugols solution (grams modification) 1 Dropper bottle containing safranin dye 1 Dropper bottle containing acetone-alcohol 1 Squeeze bottle containing tap water Bibulous paper NAS (culture 1 A) 24 hour culture	Std. Meth. 14:918-919 III.D.4 (p. 45)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedures (Continued)		1b. Twenty-four hour culture is recommended for preparation. Older cultures can give erroneous results.	
	2. Prepare glass slide.	2a. Must be clean. 2b. Helpful to clean with alcohol, distilled water, and lens tissue. 2c. Place a drop of distilled water about 1 inch from end of slide.	
	3. Obtain culture (NAS) sample and place on slide.	3a. Culture must be obtained with a flamed, air-cooled needle. NAS then is stored in refrigerator for possible need. 3b. Screw cap tube handled asceptically: * Flamed top of tube * Sterile needle * Cap carefully handled and returned to tube promptly 3c. Only minute amount of culture necessary. Large amounts can cause staining problems. 3d. Place culture from needle into water droplet and mix well while extending the droplet size to about a 1" x 1/2" area.	
	4. Prepare culture for staining procedure.	4a. Allow smear to air-dry completely and then heat fix by passing slide through the gas flame briefly back-and-forth for a heat exposure of about two seconds.	
	5. Stain culture with reagents on the side of the slide with the culture.	5a. Flood the slide with ammonium oxalate-crystal violet dye. 5b. Allow to cover culture area for 1 minute. 5c. Wash slide gently with tap water. 5d. Apply Lugols-iodine solution to culture area. 5e. Allow to cover culture area for 1 minute.	Std. Meth. 14:918-919

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedures (Continued)		5f. Wash slide gently with tap water. 5g. Apply acetone-alcohol solution to culture area. Hold slide and allow solution to flow across smear until stain is no longer being removed:  5h. Do not prolong this alcohol contact period (discolorization step) as the results may be erroneous. Some authorities suggest 10-15 seconds maximum. 5i. Wash slide gently with tap water. 5j. Apply Safranin solution (counterstain) for 15 seconds and then wash gently with tap water. 5k. Blot slide gently with bibulous paper using care not to rub culture area during procedure. 5l. Identify slide to conform to proper culture being examined. Use of a slide label is convenient (label 312, 1 A as per our example).	
	6. Examine slide microscopically.	6a. If desired, slide can be retained for later examination. If the lactose (LLSTB) broth remains negative for the culture (48 ± 3 hours), the slide need not be examined as the culture is not a coliform. 6b. Become acquainted with microscope from manufacturer's literature or individual acquainted with same.	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

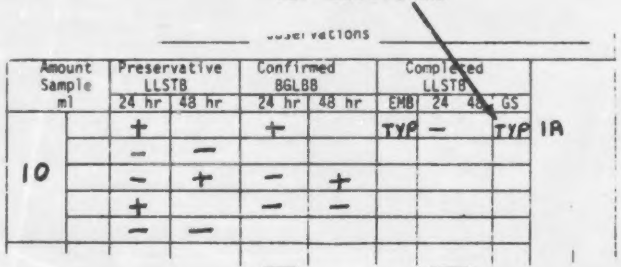
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedures (Continued)			
		6c. If examination is desired, place the slide, culture side up, on the microscope stage of a microscope equipped for oil immersion examination.	See V.D.4.6 for microscope nomenclature
		6d. Place a drop of a suitable bacteriological immersion oil on the area to be viewed (culture smear).	V.D.4.6.6d (p. 45)
		6e. The proper objective is positioned for oil-immersion (usually labeled oil and having X 97 or X 100 magnification).	
		 X20 ACHRO X100/1·3 OIL/160 TYPICAL OIL OBJECTIVE OIL IMMERSION OBJECTIVE THE OBJECTIVES SCREW INTO THE TURRET. (TURN AND LOCK FOR SELECTION)	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedures (Continued)		6f. Turn ON lighting system. Light will be directed to reflect off the plane side of a mirror through a condenser assembly and up through the stage (mirror assembly may be external or internal).	
		6g. With the illumination system correctly set up, rack down (or the stage UP on some models) until the oil-immersion lens touches and disperses the oil.	V.D.4.6.6g (p. 45)
		6h. Rack down gently with the coarse control (lens and slide will move towards each other) while looking down the microscope (into the eyepiece) until the image begins to come into focus.	
		6i. Obtain final sharp image using the fine-focus control.	
5. Gram-Stain Examination and Recording	1. Examine stained preparation for type/types of bacteria.	1a. Gram-negative bacteria (typical of coliforms) will be red or pink colorations.	
		1b. Gram-positive bacteria (NOT coliforms) will be blue-to-purple in color.	
		1c. Mixed cultures will show mixtures of the above and will immediately call for the re-isolation of pure culture on another EMB agar plate from the saved nutrient agar slant. Discard the LLSTB tube as it has no interpretative value being a mixed culture. Repeat procedures as before.	
		1d. If too large of a sample was transferred to the slide for staining, some areas of matted, numerous bacterial cells could produce areas where dyes could not either penetrate or be washed away. Recommend another smear to be made.	
	2. Record gram-stain data.	2a. Assume that, for our example, that only gram-negative organisms were observed during microscopic examination (culture 312 1 A).	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedures (Continued)		2b. Enter observation in proper place on data sheet:	

Indicates typical culture for coliforms

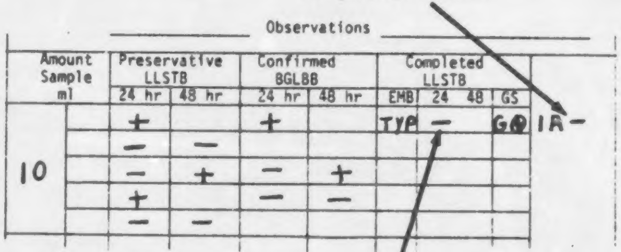


Amount Sample ml		Observations Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24	Completed LLSTB 48	GS	
10		+		+		TYP	−		TYP	IA
		−	−							
		−	+	−	+					
		+		−	−					
		−	−							

2c. Other entries could have been MXD (mixed culture) or G⊕ (gram positive).

2d. In the case of a G⊕ entry, the culture is not a coliform and a -(negative) for the completed test:

Negative tube for completed test



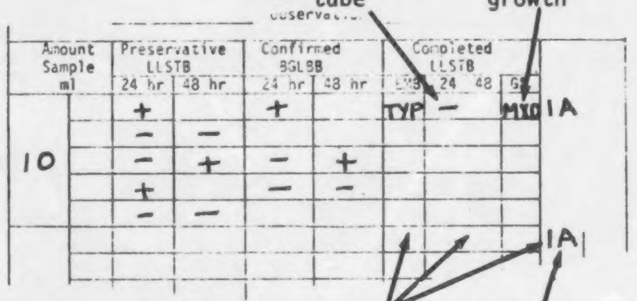
Amount Sample ml		Observations Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24	Completed LLSTB 48	GS	
10		+		+		TYP	−		G⊕	IA −
		−	−							
		−	+	−	+					
		+		−	−					
		−	−							

This tube can be discarded since it has no further relevance.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedures (Continued)		2e. In the case of a MXD (mixed culture) entry, the following interpretation can be made for the culture. * Since it is a mixed culture (gram ⊕ and Gram ⊖ organisms growing together), any results for the LLSTB tube could be in error. * The NAS (in refrigerator) for the culture is also mixed growth. * Reisolation of a pure culture must be made (NAS → EMB) for valid results.	

Discard this tube

Indicates mixed growth



Amount Sample ml	Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24 48	GS	
10	+		+		TYP	−	MXD	1A
	−	−						
	−	+	−	+				
	+		−	−				
	−	−						
								1A

New entries for this culture will be made here as they are observed

Indicates fresh EMB plate was made from the NAS

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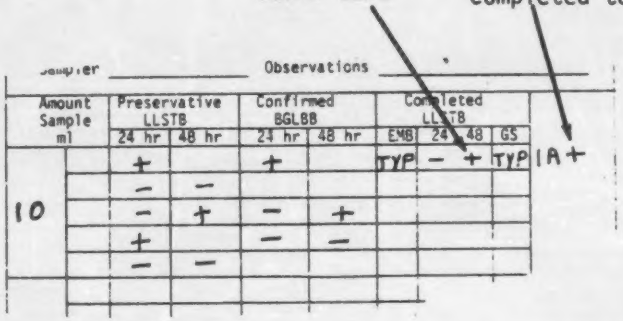
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedures (Continued) 6. Process BGLBB Positive Tube	1. Streak EMB agar plate and incubate.	1a. BGLBB tube will be labeled 312. 3 1b. Method previously described (B. First Day Procedures). 1c. Label EMB agar plate. 312 3	See data Sheet D.3.3.3b & 3c
E. Fourth-Day Procedures 1. Equipment Maintenance	1. Check, record, and adjust incubator.		
2. Lab Bench Disinfection	1. Disinfect laboratory bench.	1a. Sponge and disinfectant; paper toweling.	
3. Data Sheet Recordings	1. Locate required data sheet.	1a. Sample "312" is our example.	
	2. Remove cultures from incubator and assemble with data sheet.	2a. 1 EMB plate (24 hours old). 1 LLSTB tube (48 hours old).	See D.5.2
	3. Read and record LLSTB tube.	3a. 48 ± 3 hours incubation Tube labeled 312 1A 3c. Assume, for our example, that the tube is "positive" (contains any amount of gas in inner vial) and its recording will be:	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Fourth-Day Procedures (Continued)		+ indicates that coliforms are positive for completed test Positive LLSTB tube  **Interpretation for First Row** Coliforms present since: Gram negative non-spore forming rods which fermented lactose have been demonstrated.	
	4. Inspect and process EMB agar plate.	4a. Twenty-four hours incubation for plate labeled: 312 3	
		4b. Assume that the plate shows all colonies which are Atp (Atypical) and, therefore, two cultures must be processed:	(Previously described in Section C)

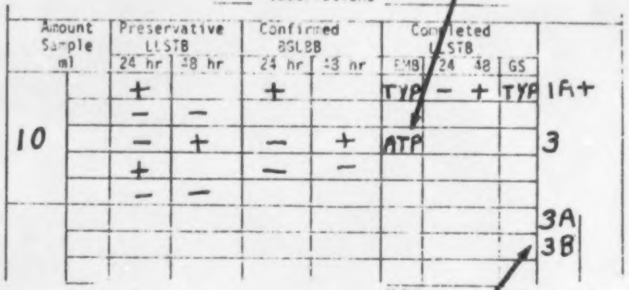
Sampler ________ Observations ________

Amount Sample ml		Preservative LLSTB 24 hr	48 hr	Confirmed BGLBB 24 hr	48 hr	Completed LLSTB EMB	24	48	GS	
10		+		+		TYP	−	+	TYP	IA +
		−	−							
		−	+	−	+					
		+		−	−					
		−	−							

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Fourth-Day Procedures (Continued)		See recording sheet below.	
F. Fifth-Day Procedures 1. Completed Test Procedure Fermentation	1. Complete test procedures.	1a. To save repetitive step procedures, the final recordings are shown below:	(Procedures as previously described)

E. Fourth-Day Procedures (Continued)

All colonies atypical



Observations

Amount Sample ml	Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24	Completed LLSTB 48	GS	
10	+		+		TYP	−	+	TYP	1A+
	−	−							
	−	+	−	+	ATP				3
	+		−	−					
	−	−							
									3A
									3B

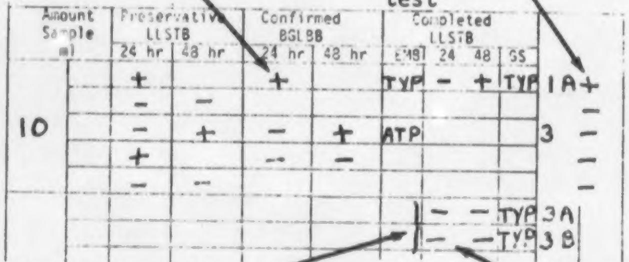
"A" and "B" indicate that 2 colonies have been picked and processed

F. Fifth-Day Procedures

1a. To save repetitive step procedures, the final recordings are shown below:

Note: 2 of 5 positive in confirmed test

Note: 1 of 5 positive in completed test

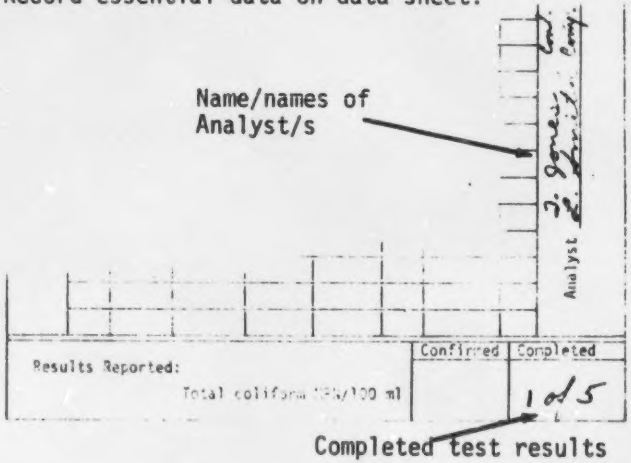


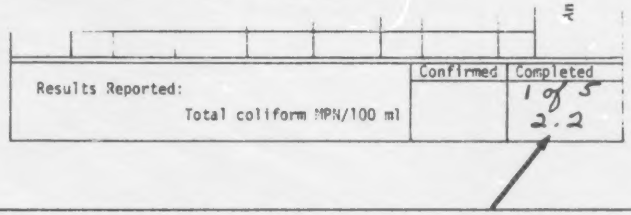
Amount Sample ml	Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24	Completed LLSTB 48	GS	
10	+		+		TYP	−	+	TYP	1A +
	−	−							−
	−	+	−	+	ATP				3 −
	+		−	−					−
	−	−							−
						−	−	TYP	3A
						−	−	TYP	3B

Both non-coliforms since cultures did not ferment lactose

Final recordings here. Note: would have taken 2 more days to complete (6 days)

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Fifth Day Procedures (Continued)		1b. Had one or both of the cultures checked out as coliforms, the third row would have been + and, therefore, resulted in 2 of 5 tubes positive instead of 1 of 5 in the completed test. 1c. Record essential data on data sheet.  Name/names of Analyst/s Analyst J. Jones Results Reported: Total coliform MPN/100 ml Confirmed Completed 1 of 5 Completed test results	
G. Interpretation of Test Results	1. Determine number of positives for the completed test.	1a. Results of confirmed test are not used since further and more conclusive testing has been done. 1b. Our example (F.1.1) shows 1 of 5 positive.	II.G.1.1 (p. 44)

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>G. Interpretaiton of Test Results (Continued)</td><td>2. Look up and note the MPN index from the MPN table.</td><td>2a. For the given example the location of the index is shown by the arrow.
MPN Index for Various Combinations of Positive and Negative Results when Five 10-ml Portions are Used
<table>
<tr><th>No. of Tubes Giving Positive Reactions out of 5 of 10 ml Each</th><th>MPN Index/ 100 ml</th></tr>
<tr><td>0</td><td><2.2</td></tr>
<tr><td>1</td><td>2.2 ←</td></tr>
<tr><td>2</td><td>5.1</td></tr>
<tr><td>3</td><td>9.2</td></tr>
<tr><td>4</td><td>16.</td></tr>
<tr><td>5</td><td>>16.</td></tr>
</table></td><td>Std. Meth. 14: 923</td></tr>
<tr><td></td><td>3. Record the calculated total coliforms per 100 ml on the data sheet.</td><td>3a. Value is direct index if, as our example, 10 ml portions were used. If 100 ml portions were used, the number is 1/10 of the index (or .2 instead of 2.2 for our example).
3b. Record under Completed Test:

<table>
<tr><th></th><th>Confirmed</th><th>Completed</th></tr>
<tr><td>Results Reported:
Total coliform MPN/100 ml</td><td></td><td>1 of 5
2.2</td></tr>
</table></td><td></td></tr>
<tr><td>H. Reporting of Results</td><td>1. Report results as prescribed under regulatory requirements.</td><td></td><td></td></tr>
</table>

WATER MONITORING PROCEDURE: Completed Test for the MPN Method for Drinking Water

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II*	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

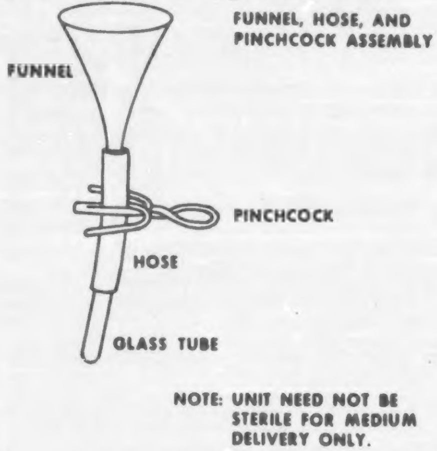
Training Guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
G.1.1	For purely qualitative aspects of testing for indicator organisms, it is convenient to consider the tests applied to one sample portion, inoculated into a tube of culture medium, and the follow-up examinations and tests on results of the original inoculation. Results of testing procedures are definite: positive (presence of the organism - group demonstrated) or negative (presence of the organism - group not demonstrated). The combination of results is used in an application of probability mathematics to secure a single MPN value for the sample. The MPN value for a given sample is obtained through the use of MPN tables. Standard practice in drinking water tests is to plant 5 tubes each containing 10 ml of sample (some organizations utilize 100 ml portions into each of 5 bottles containing increased strength medium).	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.4.1b	A colony is defined a discrete growth occurring at least 0.5 cm (approximately .2 inch) from any other growths. Such growths represent a large number of developmental successions from an original viable cell and therefore can be considered a "pure" culture. All organisms from pure cultures will exhibit the same characteristics when subjected to standard bacteriological testing.	
D.4	A gram staining procedure, in general, separates bacteria into two categories, gram positive (blue coloration) or gram negative (red coloration). Its usefullness to the coliform testing procedure is due to the fact that part of the coliform definition indicates that "gram negative, non-spore forming rods" are necessary, and, in addition, no gram positive organism must be present since some of these organisms can act synergistically (in conjunction with other non-coliforms) to produce a false positive result (gas production in lactose) which neither could manage independently. It is desirable to use known pure cultures of both a gram positive (staphylococcus, bacillus, etc.) and a gram negative (proteus, enterobacter, etc.) as controls for the staining procedure.	

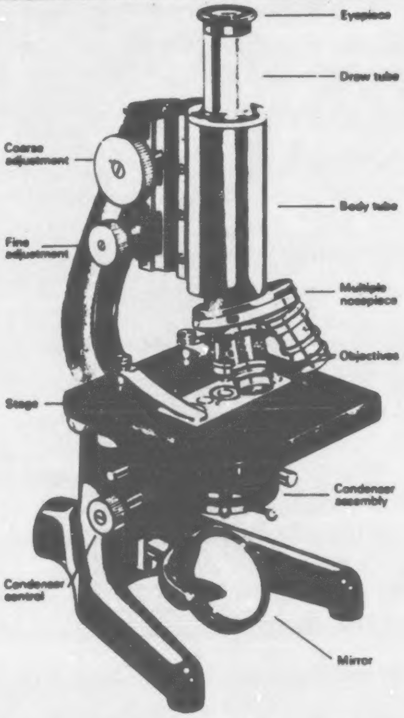
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1	Incubator must be of sufficient size for daily work load without causing crowding of tubes to be incubated. Considerations for choice of incubator type must relate to reliability of operation and not to cost or attractiveness of equipment.	
A.1.1	Incubator should be kept out of drafts or direct sunlight in order to prevent temperature inside the incubator from changing outside the temperature range specified (35° ± 0.5°C). Power supply should be selected so that there will not be too many pieces of equipment on the same circuit. Otherwise, circuits will be blown repeatedly.	Standard Methods for the Examination of Water and Wastewater, 14th ed. (1975 APHA, WPCF, AWWA, p. 880 (Hereafter referred to as: Std. Meth. 14: (page no.)
A.1.2	Mercury bulb thermometer usually used in most incubators. Recording thermometer is acceptable, but, it should be calibrated against a mercury bulb thermometer which has been certified by National Bureau of Standards. The NBS certified thermometer always should be used with its certificate and correction chart.	
A.1.3	Saturated relative humidity is required in order to make the incubation more efficient (heat is transferred to cultures faster than in a dry incubator). Furthermore, culture medium may evaporate too fast in a dry incubator.	
A.1.5	Allow enough time after each readjustment to permit the incubator to stabilize before making a new adjustment. At least one hour is suggested.	
A.1.6	Incubator temperature can be held to much closer adjustment if operated continuously. Temperature records should be kept in some form of permanent record. A temperature record book is suggested with daily recording of values. If a recording thermometer is used, the charts may be kept as permanent record; if so, be sure that the charts are properly labeled to identify the incubator and the period covered. Uniform temperature (35°C ± 0.5°C) is to be maintained on shelves in use.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.2.1-5	Since electric sterilizer will be operated intermittently, care should be taken that it is on a circuit which will not be overloaded when it is turned on. A time and temperature record is maintained for each sterilization cycle. Temperature recordings can be retained for records.	Std. Meth. 14:881
A.3.1	Autoclaves differ greatly in design and in method of operation. Some are almost like home-style pressure cookers; others are almost fully automatic. This is a subject which requires separate instruction; and should be related to the exact make and model of equipment you will use in your own laboratory. Vertical autoclaves and household pressure cookers may be used in emergency service if equipped with pressure gages and thermometers with bulbs positioned 1 inch above the water level. However, they are not to be considered the equivalent of the general purpose steam sterilizer recommended for permanent laboratory facilities. Their small size is inadequate for large-volume work loads, and they can be difficult to regulate. The following requirements must be met regarding autoclaves or sterilizing units: a. Reaches sterilization temperature (121°C), maintains 121°C during sterilization cycle, and requires no more than 45 minutes for a complete cycle. b. Pressure and temperature gages on exhaust side and an operating safety valve. c. No air bubbles produced in fermentation vials during depressurization. d. Record maintained on time and temperature for each sterilization cycle.	Std. Meth. 14:881
A.4.1-2	Distilled water in a bacteriological laboratory must not contain substances which will prevent any bacteria from growing in culture medium in which the distilled water is used or will be highly nutritive. There are procedures for testing quality of distilled water; but these should be undertaken only by professional bacteriologists or in laboratories where this is done regularly. Use only glass stills or block tin lined stills.	Std. Meth. 14:645-649 14:888-891

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.5.1	pH Meter: See cited reference.	Std. Meth. 14:882
A.6.1-4a	Glassware: See cited reference on pipets and graduated cylinders, media utensils, bottles.	Std. Meth. 14:882-885
A.6.1-4b	Glassware can be checked for bacteriostatic or inhibitory residues by a bacteriological test procedure which, like the distilled water suitability test, should be undertaken only by professional bacteriologists or in laboratories where this test is done on a regular basis.	
A.7.3	 FUNNEL, HOSE, AND PINCHCOCK ASSEMBLY FUNNEL PINCHCOCK HOSE GLASS TUBE NOTE: UNIT NEED NOT BE STERILE FOR MEDIUM DELIVERY ONLY.	
A.8.2c	Some workers prefer to utilize a magnetic whirl bar and hot plate arrangement. This is acceptable and will require no agitation until the medium is NEAR BOILING at which time the whirling action should be terminated and the medium gently swirled by hand and the flask monitored for boiling.	
B.6.5	Alternately, it is authorized to use an "incoulation stick" for transfers and plate streaking. A precisely sized and sterilized stick is intended for a one-time use and, if used, eliminates the need for a burner during the transfer procedure. Of course, several will have to be used during the streaking process since a "sterile" one is required during the streaking carryover to sterile surfaces. Also available are resterilized loops used once, resterilized, and available for future transfers.	Std. Meth. 14:917 Std. Meth. 14:883-884

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

D.4.6



D.4.6.6d Some manufacturers specify that the upper most lens of the condenser assembly also be coated with a drop of oil prior to placing the slide on the stage. In effect, this would be "sandwiching" the slide between two oil interfaces through which the light must pass.

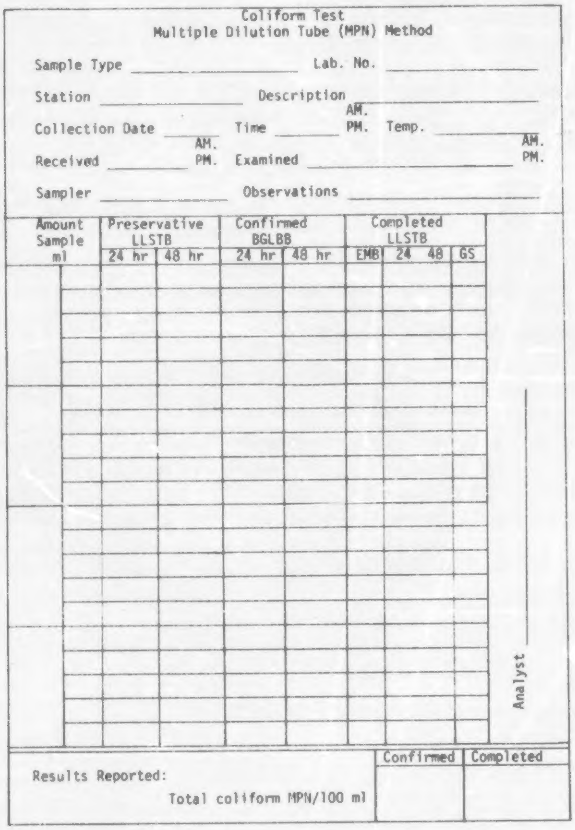
D.4.6.6g It is extremely important to properly set up the illumination system for proper results.

Procedures vary according to the type of illumination provided, the type of diaphragm used, and the controls provided by the particular microscope. Final results would give, if accomplished correctly: correct lighting from the light source; centrally placed optimal lighting; and a sharply focussed image.

TRAINING GUIDE NOTE

REFERENCES/RESOURCES

B.2 There is no such thing as a "standard data sheet for bacteriological tests. A simplified sheet is shown below:



Coliform Test
Multiple Dilution Tube (MPN) Method

Sample Type ______________ Lab. No. ______________
Station ______________ Description ______________
Collection Date ______ Time ______ AM. PM. Temp. ______
Received ______ AM. PM. Examined ______________ AM. PM.
Sampler ______________ Observations ______________

Amount Sample ml	Preservative LLSTB		Confirmed BGLBB		Completed LLSTB			
	24 hr	48 hr	24 hr	48 hr	EMB	24	48	GS

Analyst ______________

Results Reported:
Total coliform MPN/100 ml

Confirmed	Completed

Note: This data sheet could be used only to test to the confirmed stage and not proceed to the completed stage so that it can serve for a dual purpose.

B.6.6 There is no standardized way to accomplish a streak plate in order to isolate pure cultures. Some workers prefer to carry the streaks around the plate several more times with its attendant loop sterilizing sequencing between each of the streakings. Others prefer to use a specially made petri dish which features a center partition which "halves" the dish allowing two separate cultures to be cultivated. These modifications, and others, are not deviations since the only consideration which matters is that a pure culture is available for further testing.

WATER MONITORING PROCEDURES: Completed Test for the MPN Method for Drinking Water

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This outline was prepared by: Rocco Russomanno, Microbiologist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

TOTAL COLIFORM TEST FOR DRINKING WATER BY THE MEMBRANE FILTER METHOD

as applied in

DRINKING WATER TREATMENT FACILITIES
and in the
DISTRIBUTION SYSTEMS OF DRINKING WATER TREATMENT FACILITIES

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U. S. Environmental Protection Agency

BA.MET.lab.WMP.2a.10.78

EPA 600/8-78-008, May 1978
Manual for the Interim Certification of Laboratories Involved in Analyzing Public Drinking Water Supplies - Criteria & Procedures

Total Coliform Test for Drinking Water by the Membrane Filter Method[1]

The required procedures, which are mandatory, are described in the 13th Edition of "Standard Methods": single step or enrichment standard total coliform membrane filter procedure (p. 679-683). Tentative methods are not acceptable. All other procedures are considered alternative analytical techniques as described in section 141.27 of the National Interim Primary Drinking Water Regulations. Application for the use of alternative methods may require acceptable comparability data.

Membrane Filtration Equipment

Units must be made of stainless steel, glass, or autoclavable plastic. Equipment must not leak and must be uncorroded.

Field equipment is acceptable for coliform detection only when standard laboratory MF procedures are followed.

Membrane filter assemblies (wrapped) and sterilized at 121°C/30 minutes.

Membrane Filters and Pads

Membrane filters must be manufactured from cellulose ester materials, white, grid-marked, 47-mm diameter, 0.45 μm pore size. Another pore size may be used if the manufacturer gives performance data equal to or better than the 0.45 μm membrane filter. Membranes and pads must be autoclavable or presterilized (autoclaved at 121°C for 10 minutes with fast exhaust). Membrane filters used must be those recommended by the manufacturer for water analysis. The recommendation must be based on data relating to ink toxicity, recovery, retention, and absence of growth-promoting substances.

Total Coliform Media

M-Endo broth, M-Endo agar, or Les Endo agar used in a single step procedure; final pH 7.2 ± 0.2; total incubation time 22 to 24 hours at 35° ± 0.5°C.

In two-step Les M-Endo procedure, MF incubated on lauryl-tryptose-broth-saturated absorbent pad for 1.5 to 2 hours at 3.5° ± 0.5°C; then on M-Endo broth or Les Endo agar for 20 to 22 hours at 35° ± 0.5°C.

Reconstituted in laboratory pure water containing 2 percent ethanol (not denatured).

The membrane filter broth and agar media must be heated in a boiling water bath until completely dissolved.

Membrane filter (MF) broths must be stored and refrigerated no longer than 96 hours. MF agar media must be stored, refrigerated and used within 2 weeks if prepared in tight-fitting dishes.

Ampouled media must be stored at 1° to 4.4°C (34° to 40°F); time must be limited to manufacturer's expiration date.

MF Culture Dishes

Sterile tight or loose-lid plastic culture dishes or loose-lid glass culture dishes must be used. For loose-lid culture dishes, relative humidity in the incubator must be at least 90 percent.

Culture dish containers, if used, must be aluminum or stainless steel; or dishes may be wrapped in heavy aluminum foil or char-resistant paper.

Open packs of disposable sterile culture dishes must be resealed between uses.

Stock buffer solution must be prepared according to "Standard Methods" using laboratory pure water adjusted to pH 7.2. Stock buffer must be autoclaved or filter-sterilized, labeled, dated, and stored at 1° to 4.4°C. The stored buffer solution must be free of turbidity.

Rinse and dilution water must be prepared by adding 1.25 ml of stock buffer solution per liter of laboratory pure water. Final pH must be 7.2 ± 0.1.

Rinse water volumes of 500 ml to 1,000 ml sterilized at 121°C/45 minutes.

Rinse water in excess of 1,000 ml sterilized at 121°C/time adjusted for volume; check for sterility.

Filtration assembly sterile at start of each series and must be sterilized between sample filtration series. A filtration series ends when 30 minutes or longer elapse between sample filtrations.

At least 2 minutes of UV light or boiling water may be used on membrane filter assembly to prevent bacterial carry-over between filtrations (optional).

Absorbent pads saturated with medium, excess discarded; or 4.0 ml of agar medium can be used per culture dish instead of a pad.

Sample shaken vigorously immediately before test.

Test sample portions measured and not less than 100 ml.

Funnel rinsed at least twice with 20- to 30-ml portions of sterile buffered water.

MF removed with sterile forceps grasping area outside effective filtering area.

MF rolled onto medium pad or agar so air bubbles are not trapped.

Low power magnification device with fluorescent light positioned for maximum sheen visibility.

Total coliform count calculated in density per 100 ml.

A start and finish MF control test (rinse water, medium, and supplies) must be conducted for each filtration series. If sterile controls indicate contamination, all data on samples affected must be rejected and a request made for immediate resampling of those waters involved in the laboratory error.

The following rules for reporting any problem with MF results must be observed:

- Confluent growth: Growth (with or without discrete sheen colonies) covering the entire filtration area of the membrane. Results are reported as "confluent growth per 100 ml, with (or without) coliforms," and a new sample requested.
- TNTC (Too numerous to count): The total number of bacterial colonies on the membrane is too numerous (usually greater than 200 total colonies), not sufficiently distinct, or both. An accurate count cannot be made. Results are reported as "TNTC per 100 ml, with (or without) coliforms," and a new sample requested.
- Confluent growth and TNTC: A new sample must be requested, and the sample volumes filtered must be adjusted to apply the MF procedure; otherwise the MPN procedure must be used.

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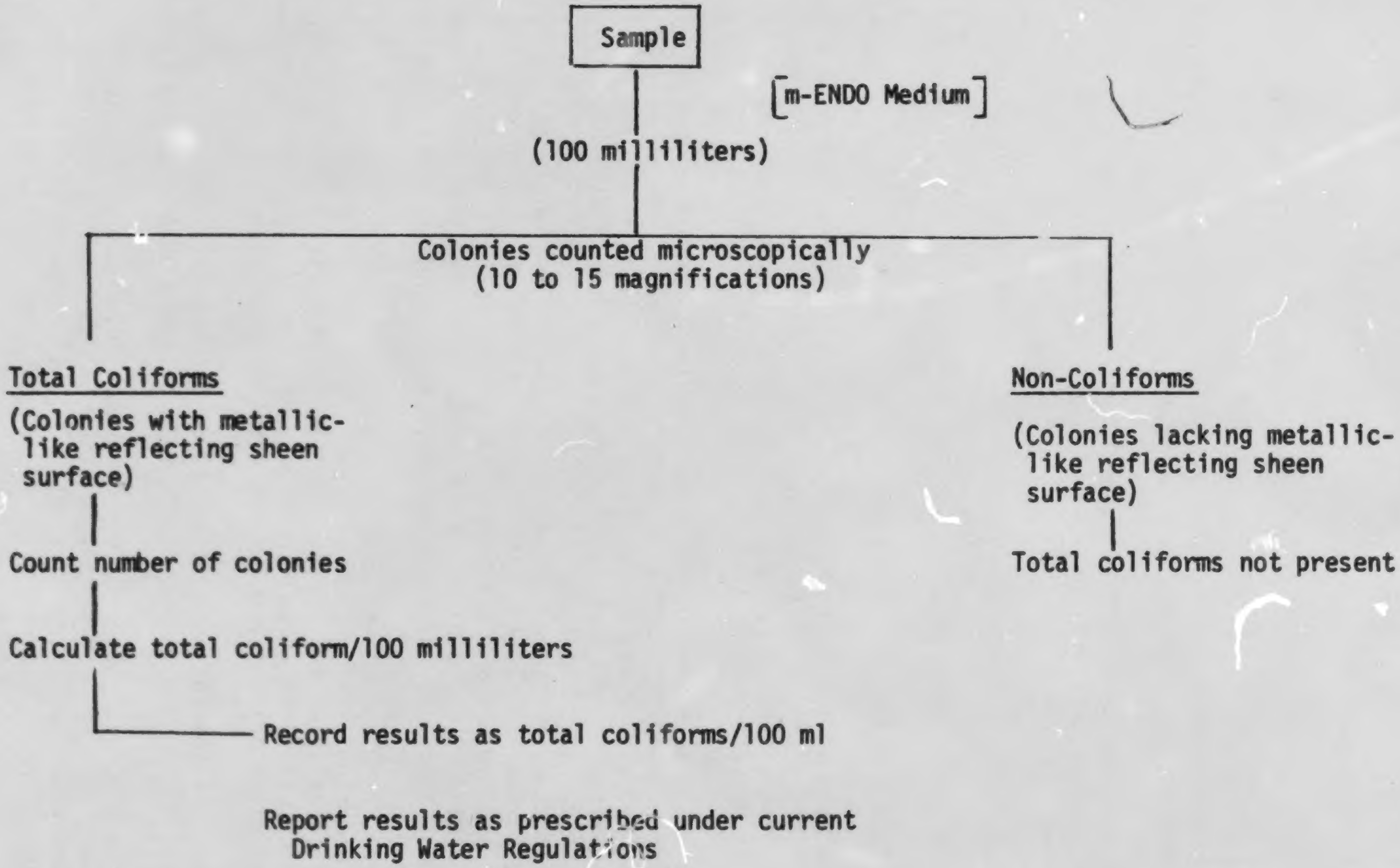
[1] MINIMUM REQUIREMENTS except where indicated as OPTIONAL.

TOTAL COLIFORM TEST

Membrane Filter Method

Flow Sheet


Sample
[m-ENDO Medium]
(100 milliliters)
Colonies counted microscopically (10 to 15 magnifications)
Total Coliforms
(Colonies with metallic-like reflecting sheen surface)
Count number of colonies
Calculate total coliform/100 milliliters
Record results as total coliforms/100 ml
Report results as prescribed under current Drinking Water Regulations
Non-Coliforms
(Colonies lacking metallic-like reflecting sheen surface)
Total coliforms not present

(IMPORTANT: A total of 200 or more colonies of any type invalidates the sample for counting purposes).

WATER MONITORING PROCEDURE: Total Coliform Test for Drinking Water by the Membrane Filter Method

1. Analysis Objectives:

In drinking water monitoring, the application of this methodology can be for any of the following:

a. Test parameter for the conduction of a sanitary survey during an on-site review of the water source.

b. Special purpose testing such as those taken to determine whether disinfection practices following pipe placement, replacement, or repair have been sufficient.

c. Check tests following unsatisfactory coliform results, and,

d. Monitoring potable water supplies.

2. Brief description of analysis:

A standard portion* of 100 milliliters is filtered through a membrane filter contained within a filtering apparatus. Bacteria in the sample portion are held on the upper surface of the membrane, while the water passes through and is discarded.

After several rinses of the funnel of the filtering apparatus with sterile buffered distilled water, the membrane filter is placed on a paper pad saturated with a medium called m-ENDO Broth within a petri dish. The closed and inverted petri dish is now incubated within a high humidity incubator set at 35° C ± 0.5° C for an incubation period of 22-24 hours. On this medium, coliform bacteria will grow and develop a golden metallic sheen-like surface on the colonies. Colonies lacking this characteristic reflective surface are not considered as coliforms. This distinctive surface sheen may appear at the center, edges, or all-over the colony. At times it can form as flecks or particles of sheen throughout or partially covering the colony.

The membrane is inspected with the aid of a microscope or lens having a magnification of 10X or 15X under reflective lighting from a fluorescent source. Coliform colonies, if any, are counted and a calculation made in order to report total coliforms per 100 milliliters.

Analytical Method: Standard Methods for the Examination of Water and Wastewater, 14th Edition, 1975, Pg. 928 ff.

*Procedures will be hereafter described for monitoring a drinking water sample and not concerned with other types of samples which require similar but differing instructions (i.e., more sample portions, more petri dishes, discrimination of which plates to count, etc.).

WATER MONITORING PROCEDURE: Total Coliform Test for Drinking Water by the Membrane Filter Method

Equipment and Supply Requirements

A. Capital Equipment:

1. Autocalve, steam, providing uniform temperatures up to and including 121° C and equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperatures within 30 minutes. (Alternately, a suitable pressure cooker is acceptable-see Standard Methods for particulars.)
2. Incubator, air, providing uniform and constant temperature of 35° C ±0.5°C and having an atmosphere of at least 90% relative humidity.
3. Oven, hot-air, providing uniform temperatures within the range of 160 - 180° C.
4. Apparatus, water distillation, distilled water product suitable for bacteriological operations. (Alternately, a suitable source is permissible.)
5. Microscope, stereoscopic, 10X to 15X magnification with fluorescent lighting mandatory. (Alternately, a suitable magnifying lens with fluorescent lamp is acceptable.)
6. Refrigerator, set for less than 10° C but above the freezing temperature.
7. Vacuum Source, preferably a pump assembly with suitable hoses and shut-off valve provided. (Alternately, an aspirator or hand pump with the same provisions are acceptable.)
8. Balance, analytical, sensitivity of 1 mg.
9. Gas Source, suitable for burner. (Alternately, an alcohol lamp can be used.)

B. Reusable Supplies:

1. Apron, suitable for laboratory operations.
2. Bottle, sample, of sufficient size for standard sample, preferably of 250 ml, wide-mouth, glass stopper, with tag. (Alternately, 120 ml size.)
3. Bottle, squeeze type, containing disinfecting solution.
4. Burner, gas, suitable for laboratory operations with connecting hose.
5. Thermometer, NBS (or NBS calibrated), functions within 20° - 60° C range with individual markings of 0.2° C or less.
6. Thermometer, NBS (or NBS calibrated), functions within 150° - 190° C range with individual markings of 1° C.
7. Filtration Unit, MF, a seamless funnel attached to a receptacle bearing a porous plate (screen, porous disc, etc.) and constructed from stainless steel, glass, porcelain, plastic, or other suitable material.
8. Hot Plate, controllable heat range up to the 100° C range.
9. Balance, trip, sensitivity of 0.1 gram at a load of 150 grams, with appropriate weights.
10. Meter, pH, accurate to within 0.1 pH unit, with suitable standard pH reference solution(s).
11. Can, pipet, non-toxic and sterilizable material (if pre-sterilized disposable type pipets are used, this item is unnecessary).
12. Pan, discard, receives contaminated material and pipets and contains disinfectant, of sufficient length to receive pipets placed horizontally.
13. Cylinder, graduated, 500 ml, 100 ml and 25 ml size.

14. Blank, dilution water, 99 ml.
15. Pipets, microbiological, 5.0 ml, with 0.1 ml graduations, sterile cotton plugged, glass or disposable types (the disposable types are for one time use and may be glass or plastic).
16. Pipets, microbiological, 1.0 ml, with 0.1 graduations, sterile cotton plugged, glass or disposable types (the disposable types are for one time use and may be glass or plastic).
17. Pipets, microbiological, 10 ml, with 1 ml graduations, sterile, cotton plugged, glass or disposable types (the disposable types are for one time use and may be glass or plastic).
18. Beaker, 50 ml (for measuring pH).
19. Flask, volumetric, 1 liter capacity (for stock solution of phosphate buffer).
20. Flask, Erlenmeyer, 500 ml capacity (for holding buffered distilled rinse water).
21. Flask, sidearm, 1 liter size for reservoir of MF apparatus; proper size bored, rubber stopper is needed to connect MF filtration flask to flask and hose required to vacuum source (must be rigid enough to avoid collapse under vacuum and flexible enough to be controlled by pinch clamp) pinch clamp - vacuum control.
22. Flask, Erlenmeyer, 50 ml (for preparing m-ENDO medium).
23. Forceps, curved end, round tip.
24. Bottle, small, Methanol or Ethanol volume to cover ends of forceps.
25. Sponge, small, to spread and wipe germicide.
26. Desiccator, media storage, ideally opaque or darkened and containing desiccating agent to remove moisture.

C. Consumable Supplies:

1. Dish, petri, disposable, tight fitting plastic, 50 x 12 mm, sterile.
2. m-ENDO Broth, medium, dehydrated, total coliform. Distributors, Difco, BBL, or other equivalent preparation.
3. Pencil, wax, recommended of soft wax equivalent to Blaisdell 169T.
4. Tags, bottle marking.
5. Glass Wool.
6. Cotton, non-absorbent.
7. Paper, Kraft wrapping.
8. Foil, aluminum, heavy duty.
9. Matches or striker.
10. Towels, paper.
11. Detergent, non-toxic, laboratory cleaning.
12. Data Sheet, as required by analyst's agency.
13. Filter, membrane, 47mm, 0.45 µm pore size, white, grid marked, sterile.
14. Pad, absorbent, 48 mm, sterile (usually included with membrane packet).
15. Potassium Dihydrogen Phosphate (KH_2PO_4), recommended 1/4 lb.
16. Sodium Thiosulphate ($Na_2S_2O_3SH_2O$).
17. Disinfectant, for bench tops and decontaminating purposes, bleach of household strength and prepared according to label directions.
18. Sodium Hydroxide (NaOH), 1N.
19. Distilled Water, suitable for bacteriological operations. Obtainable from distillation apparatus (see Capital Equipment) or suitable source of supply.

20. Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$).
21. Ethanol, 95%.

Item needs in quantities or required size or space allowances cannot be specified, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of Standard Methods for the Examination of Water and Wastewater.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures		Aa. All pretest procedures completed before starting other first-day procedures.	
1. Incubator Setup, Adjustment (35° C ± 0.5° C)	1. Place incubator in permanent location.	1a. Floor location for large unit or table or bench for smaller units. 1b. Out of drafts or place in which it will be in direct sunlight part of day. 1c. Location convenient to laboratory operations. 1d. Convenient source of electric power with a separate circuit, if possible.	V.A.1.1 (p. 5-32)
	2. Provide a saturated humidity within incubator.	2a. Check manufaturer's handbook for maintenance of humidification system, if installed. 2b. If humidifier system not installed within incubator, place beakers or trays containing distilled water on shelves to provide relative humidity of at least 90 percent during operating temperatures.	III.A.1.2b. (p. 5-31)
	3. Install thermometer.	3a. Functions at least in 30° - 40° C range. Meets NBS standards. Have 0.2° C increment markings or less. 3b. Usually a corner location to prevent breakage and tip immersed in a bottle containing water, glycerin, etc. for a more stable reading. 3c. If thermometer assembly has been installed by manufacturer, check for above requirements and calibrate with NBS thermometer. Calibration may be possible by removal and testing of installed unit or by comparsion during incubator operation.	V.A.1.3 (p. 5-32)
	4. Connect incubator to electric power source and turn ON.	4a. Pilot light should come on.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for location and method of temperature adjustment. 5b. Allow about 1 hour between fine adjustment (less than 2 degrees) and immediate adjustments can be made when the calibration is greater than this amount. Temperature achievement by the setting knob will be usually indicated by either a light indication or by an alternate lighting of a "heat-ON" - "refrigerant-ON" or other arrangement depending upon the incubator type/model.	
	6. Operate incubator continuously.	6a. Operate incubator continuously unless it will be unused for a relatively long period. (2 weeks or more). 6b. Daily check of temperature required, preferably an early morning and late afternoon with a written record maintained. Adjust temperature if necessary. 6c. Check at least biweekly the humidity level of interior of incubator. Add water to humidifier unit, if applicable, or to trays placed on the shelves providing humidification by convection.	
2. Oven Sterilizer-Drier Setup, Adjustment (170° ± 1.0C)	1. Place oven sterilizer-drier in permanent location.	1a. Convenient source of electric power.	V.A.2 (p. 5-32)
	2. Connect oven/drier to power source and turn ON.	2a. Usually an indication is given that power is applied- such as an indicator light.	
	3. Install thermometer.	3a. Thermometer should read within 160-180° C range, be accurate within this interval, and be marked in 1.0° C intervals. 3b. If installed by manufacturer, ascertain if installation meets the above requirements.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	4. Adjust oven temperature to stabilize at required sterilizing temperature.	4a. 170° C is the required temperature. Arbitrarily, for this publication, a 1 degree leeway is stipulated. 4b. Manufacturer's instruction for location and method of temperature adjustment. 4c. Allow about 1 hour between fine adjustments (less than 2 degrees of desired temperature) and immediate adjustments can be made when the calibration is greater than this amount.	
3. Autoclave Setup	1. Install and operate autoclave according to manufacturer's instructions.	1a. Variable in design and operation, and unless properly operated can be dangerous. 1b. Used to sterilize objects made of or including liquids, rubber, and some plastics, and, for glassware, if desired. 1c. Operated for general sterilization at 121° C. (250° F) for a period of 15 minutes after this temperature has been attained. 1d. Sterilized media and liquids must be removed as soon as possible upon completion of sterilization from the chamber of the autoclave.	
4. Water Distillation Equipment Setup	1. Install and operate in accordance with manufacturer's instructions.	1a. Must produce water meeting quality requirements for bacteriological tests.	V.A.4 (p. 5-33)
	2. Operate as required to maintain adequate supplies of suitable distilled water.		
5. pH Meter Setup	1. Setup and operate in accordance with manufacturer's instructions.	1a. Meter must be accurate to at least 0.1 pH unit.	

WATER MONITORING PROCEDURE: Total Coliform Test for Drinking Water by the Memebrane Filter Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued) 6. Glassware Preparation	1. Clean and rinse using a suitable detergent and hot water.	1a. Nontoxic detergent must be completely removed from glassware.	
	2. Use final rinsing of deionized or distilled water.	2a. 6 to 12 successive rinsings may be required. 2b. Must produce a clean dry glassware which meets bacteriological requirements for suitability.	
7. Sample Bottle Preparation	1. Deliver 0.1 ml or 0.2 ml of 10% sodium thiosulfate solution to each sample bottle (0.1 ml to 4 oz. or 120 ml size and 0.2 ml to 6-8 oz. or 250 ml size).	1a. Bottle meets glassware requirements. 1b. Use 1 ml pipet. 1c. Provides adequate sodium thiosulfate for neutralizing chlorine in sample.	
	Sodium thiosulfate is prepared as follows:	Sodium Thiosulfate Preparation	
	* Weigh 10.0 grams of sodium thiosulfate.	1d. Use of trip balance for weighing acceptable.	
	* Dissolve in 50-60 ml of distilled water.	1e. 100 ml graduated cylinder satisfactory for volume measurements.	
	* Add distilled water to bring final volume to 100 ml.	1f. Final preparation should be labeled as 10% Sodium Thiosulfate and stored in refrigerator.	
	* Transfer to labeled bottle.		
	2. Place cover on sample bottle.		
	3. Place paper or metal foil cover over bottle cap or stopper.	3a. Protects opening of sample bottle from accidental or natural contamination.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	4. Sterilize sample bottle in autoclave or oven.	4a. In oven at 170° C for 1 hour. In autoclave at 15 pounds for 15 minutes.	
	5. Store sample bottle in clean dry place until used.		
8. Pipet Preparation	1. Insert a plug of non-absorbent cotton into mouthpiece of clean, dry pipet.	1a. Pipets which have chipped or broken tips or tops should be discarded. 1b. Cleanliness and suitability of pipets equivalent to bacteriological suitability of glassware. 1c. Cotton plug must be tight enough to prevent easy removal, either by pipeting action or by handling, and yet be loose enough to permit easy air movement through the plug. 1d. Plug protects user from ingesting sample into his mouth.	
	2. Pass plugged end of pipet quickly through burner.	2a. Removes wisps of cotton which interferes with fingertip control of pipeting action.	
	3. Insert a layer of glass wool or multi-layer of paper padding in bottom of pipet can.	3a. Protects tips from damage. 3b. Pipets can be sterilized individually, if desired, by wrapping in Kraft paper then oven sterilizing. This technique would make the use of pipet cans unnecessary.	
	4. Place pipet in pipet can with delivery tip downward and contacting glass wool or paper. Close can when full or desirable to complete preparation.	4a. Cotton-plugged mouthpiece in pipeting, is finger control end with the delivery tip on the opposite end. 4b. Approximately twenty (20) 1 ml pipets or twelve (12) 10 ml pipets will normally be accommodated in these cans. 4c. Can must be able to withstand sterilizing conditions. Toxic materials, such as copper, must not be used. Aluminum is acceptable.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	5. Sterilize pipets.	5a. At least 1 hour in oven at 170° C, or 5b. In autoclave at 15 pounds steam pressure for 15 minutes. Cans removed quickly from autoclave after sterilizing with aid of asbestos gloves and opened quickly and slightly to allow residual steam to escape for a few seconds.	
	6. Store cans in a clean dry place until needed.		
9. Blanks, Dilution Water	1. Prepare stock solution of potassium dihydrogen phosphate (KH_2PO_4) by dissolving 34.0 grams of this chemical in 500 ml of distilled water and adjusting its pH to 7.2 with 1N NaOH. Dilute to 1 liter in volumetric flask.	1a. Distilled water may be measured in 500 ml graduated cylinder. 1b. Label to show contents, identity of preparer, and date of preparation. 1c. Stored in refrigerator. 1d. Discarded if mold or turbidity appear.	
	2. Prepare stock solution of magnesium sulfate ($MgSO_4.7H_2O$) by dissolving 50 grams of this chemical in 500-600 mls of distilled water and, after complete dissolving, bring the final volume to 1 liter in a volumetric flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	3. Prepare working solution of dilution water by adding 1.25 ml of the potassium dihydrogen phosphate stock solution and 5 ml of the magnesium sulfate stock solution to each liter of distilled water to be used in the preparation of dilution water.	3a. A 10 ml or 5 ml pipet is satisfactory for delivery of both of these stock solutions provided that it has graduation marks to deliver the proper amount. Use separate pipets for each solution to prevent contamination.	
	4. Deliver enough working solution to each dilution water bottle so that after sterilization the bottle will contain 99 ± 2 ml of dilution water.	4a. Recommended that dilution water bottles have a marking at the desired 99 ml quantity. Amount to be delivered to bottle before sterilization cannot be stated exactly as evaporation is different with differing conditions and autoclaves. Ordinarily about 102 ml will be required.	
	5. Place caps on bottles loosely.		
	6. Sterilize in autoclave.	6a. 15 minutes at 121° C.	
	7. Remove from autoclave, tighten bottle caps; cool to room temperature.		
	8. Store in cool place.	8a. Dilution bottles ready for use. May be stored indefinitely. 8b. Some evaporation loss may occur in time and in these cases, sterile similarly prepared water may be added. This is why a calibrated marked bottle is desirable.	

WATER MONITORING PROCEDURE: Total Coliform Test for Drinking Water by the Membrane Filter Method

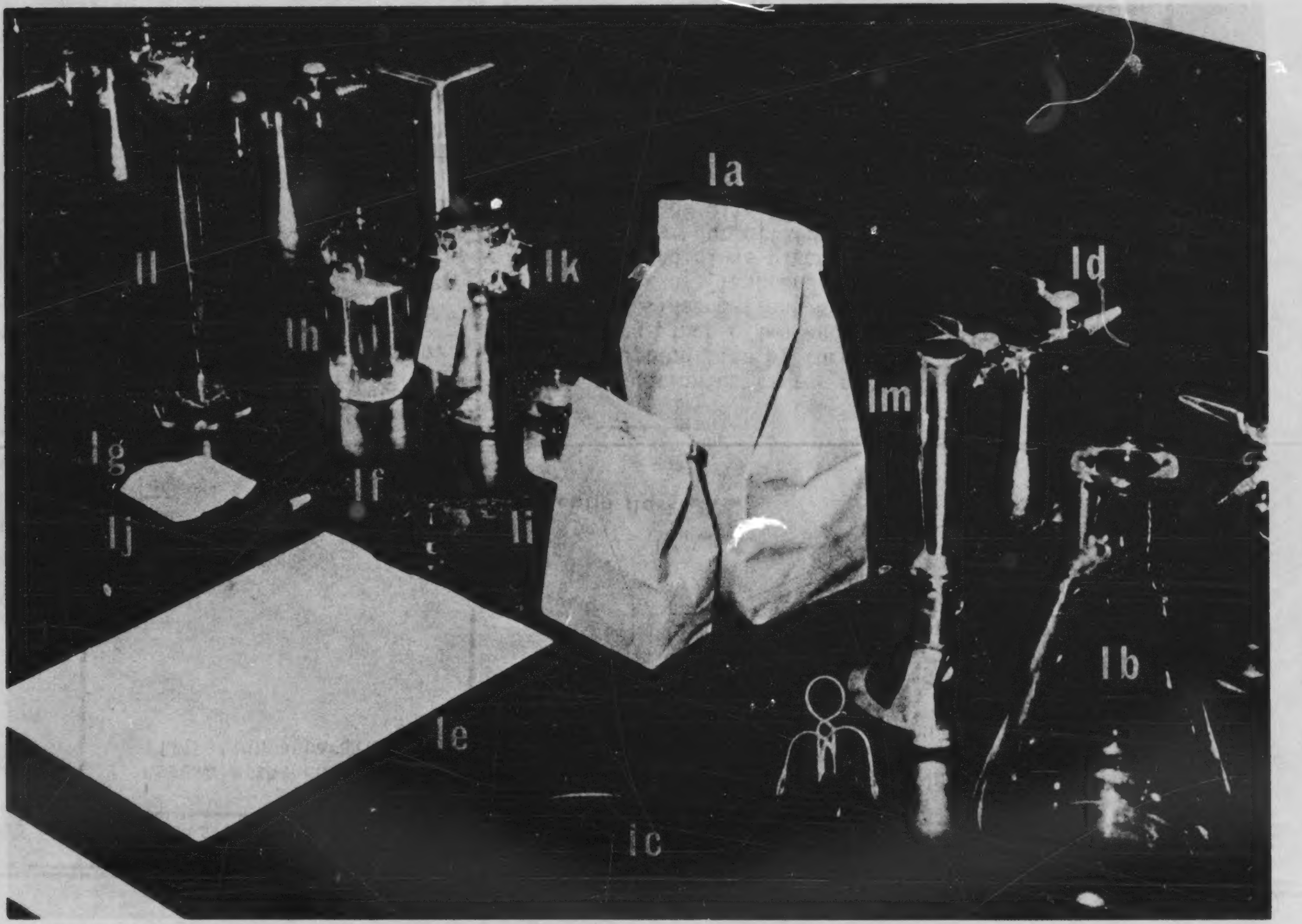
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued) 10. Preparation of Total Coliform Medium	1. Retrieve and inspect dehydrated m-ENDO broth medium.	1a. Best stored in desiccator which prevents moisture from damaging medium. 1b. Powder must be light pink without signs of hardening or color change to blood red hue.	VI.A.10.1 (p. 5-35)
	2. Weigh 1 gram of dehydrated m-ENDO broth.	2a. Sufficient for 20 ml of medium which prepares 10 petri dishes. 2b. Analytical balance having a sensitivity of 1 mg will be required. 2c. More than 2 grams being weighed can be done on less sensitive balance. This would provide more plates, but, of course, some medium can be discarded.	II.A.10.2 (p. 5-30)
	3. Place powder in a clean, dry 50 ml Erlenmeyer flask.		
	4. Prepare an alcohol-water solution as follows: a. Place 0.4 ml of ethanol in a clean, dry 25 ml graduate. b. Add distilled water to the graduate to the 20 ml mark.	4a. Graduate need not be sterile. No acceptable substitutes for ethanol. Use 1 ml pipet graduated in 0.1 ml increments. 4b. A squeeze bottle addition to the graduate makes control of the distilled water addition easier.	VI.A.10.4 (p. 5-35)
	5. Add a small amount of the ethanol-water solution to the powder in the flask (about 5 ml). Swirl flask to mix powder and then add the remainder of the water.	5a. Small addition of water makes it easier to remove powder from walls of flask.	

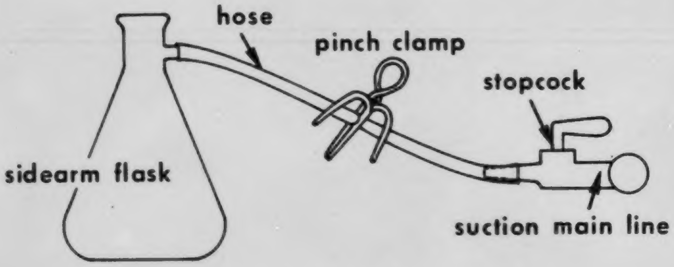
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	6. Cover top of flask with aluminum foil.	6a. Some laboratories use a cap to cover a screw-cap flask. If this is to be practiced, make sure that the cap is LOOSE when heating to relieve pressure built up during heating.	
	7. Heat flask on a hot plate set to HIGH heat range.	7a. Constant stirring is necessary to prevent charring or burning of medium.	
	8. Remove at first sign of boiling.	8a. Prolonged heating reduces selectivity of medium. 8b. Do not autoclave this medium. 8c. Medium ready for use. Can be stored in refrigerator for up to 96 hours before discarding.	
11. Preparation of m-ENDO Plate	1. Remove a sterile petri dish from its container. Loosen its cap without removal.	1a. Usually in a sleeve of pre-sterilized plastic one-time-use dishes. 1b. Laboratory resuable sterilized glass dishes can alternately be used.	
	2. Remove a sterile absorption pad from its container and place in dish. Replace cap which is still loosely fitting.	2a. Usually packaged with membrane filters, or, alternately, can be separately laboratory sterilized. 3b. Use a forceps which has been standing in a flask of alcohol and then passed quickly through a flame to remove residual alcohol.	
	3. Transfer approximately 2 ml of the m-ENDO broth to the absorption pad within the dish.	3a. Plate can be stored in refrigerator for up to 96 hours before discarding, or used immediately.	
	4. Gently tip the opened petri dish until a droplet of medium forms on the inner lower edge.	4a. A 2 ml broth addition is usually an execessive amount. 4b. Hold petri dish cover in other hand. Do not allow it to become contaminated.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	5. Gently shake out large droplet to waste. Replace cover tightly.	5a. Plate is ready for use in analysis. 5b. Keep plate from excessive exposure to light, particularly sunlight. 5c. It has been found that this procedure will invariably give an optimum amount of medium, whereas, trying to measure precisely the same amount for each plate will give less than optimum amounts more frequently. 5d. If plate is to be used within the hour, simply cover with a paper towel on the bench. If a greater time is expected, place in refrigerator until used.	
B. First Day Procedure 1. Equipment Maintenance	1. Check, record, and adjust, if necessary, the 35° C incubator.		
	2. Check, record (if done) and adjust (if necessary) the refrigerator.		
2. Sample Collection and Handling	1. Collect sample, use a grab, direct filling, or suitable device collection technique.	1a. Representative of water supply system. 1b. Leave sufficient air space in bottle to allow shaking of sample (at least 2.5 cm or 1 inch). 1c. Do not rinse bottle before collecting sample as this would cause loss of dechlorinating agent. 1d. Exercise care to prevent contamination of samples.	
	2. Apply label to bottle and enter required information.	2a. Enter required information as per agency requirements. A minimum useful amount of entries include: * name of sampler (complete name, not initials) * location/code of collection site * time of collection * chlorine residual (water before sampling) * date of collection.	

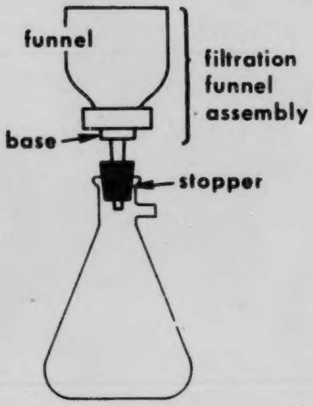
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)	3. Place bottle in closed ice chest.	3a. Use ice in chest if possible, although there is no strict requirement for this procedure. However, protect the bottle from detrimental conditions such as direct sunlight, extreme heat or cold, etc.	
	4. Transport to laboratory and dispose of sample in accordance with laboratory policies.	4a. An undesirable, but acceptable practice, is to retain the bottle for up to 30 hours prior to analysis before discarding as an unacceptable sample. Sample should be analyzed as soon as possible.	
3. Preanalysis Preparation	1. Prepare laboratory data sheet.	1a. No standard data sheet. Use form recommended by laboratory/Agency. 1b. Some of required information will be on sample label.	
	2. Disinfect laboratory bench; wipe dry.	2a. Use sponge and disinfectant; paper toweling.	
4. Equipment and Material Assembly	1. Assemble required equipment and material.	1a. Filtration funnel assembly, sterile. 1b. Side arm suction flask, 1 liter size. 1c. Hose, suction w/clamp. 1d. Vacuum source, operational. 1e. Sheet, data. 1f. Prepared m-ENDO dish. 1g. Membrane filter, sterile. 1h. Buffered distilled rinse water (about 100 ml per test). 1i. Forceps and disinfectant container (methanol). 1j. Pencil, marking. 1k. Sample bottle. 1l. Graduate, sterile, 100 ml, foil hood protected. 1m. Burner, gas, w/hose joined to gas source.	

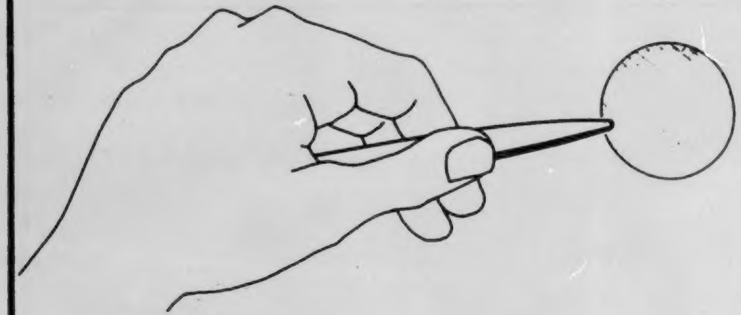
WATER MONITORING PROCEDURE: Total Coliform Test by the Membrane Filter Method

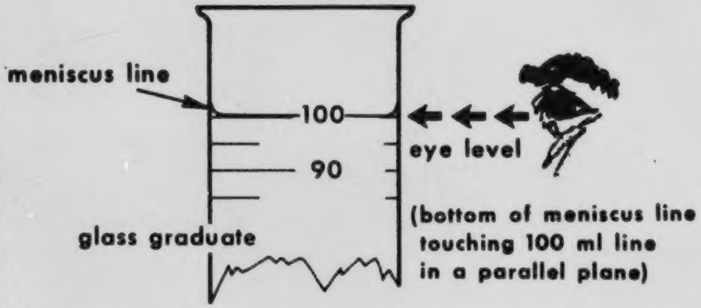


OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)	2. Assemble the units of the filtration apparatus.	2a. Suction side of apparatus may, depending on choice of equipment, consist of sidearm suction flask, suction line, pinch clamp, and suction device. Such an arrangement is shown below:  hose pinch clamp stopcock sidearm flask suction main line	V.B.4.2 (p. 5-33)
	3. Test the filtration apparatus for operation.	3a. Check suction units for cleanliness and operation: Open suction line by turning on stopcock and removing pinch clamp and check for suction at neck of sidearm flask by placing palm of hand over neck of flask and noting presence of suction. Replace and close pinch clamp and note if suction is cut off from the flask. NOTE: This test is made without the filtration funnel assembly being installed.	
	4. Assemble the units of the filtration unit assembly: Unwrap sterile funnel base from wrapping and place on base unit.	4a. The filtration unit assembly consists of a funnel and a base which should be clean, sterile, and in operational status. 4b. Manufacturers usually provide kits for maintenance of units. 4c. Do not contaminate working areas of funnel assembly (screen, inner area of funnel, funnel lip, etc.). 4d. Stopper may be retained on base of filtration unit throughout the usage and sterilization of the base.	

WATER MONITORING PROCEDURE: Total Coliform Test for Drinking Water by the Membrane Filter Method

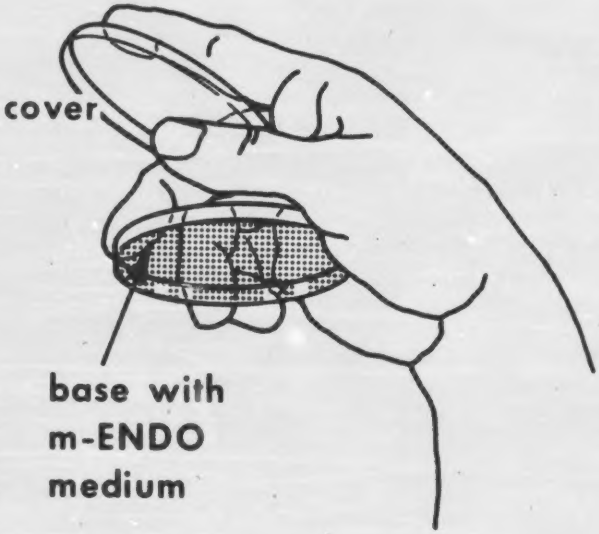
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)		4e. Units of filtration assembly are sterilized by steam after wrapping in Kraft paper or aluminum foil.  	
	5. Light burner.	5a. Some laboratories use an alcohol lamp.	
	6. Label m-ENDO plate with necessary identification markings.	6a. Conforms to data sheet.	
5. Sample Filtration	1. Place membrane filter (MF) on base of funnel unit and centered evenly on the screen assembly.	1a. Funnel top removed carefully to avoid contamination. Do not place on contaminated surface. Best to hold in hand while using forceps in other.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)		1b. MF placed grid or inked side UP. MF handled with flamed forceps and only on the membranes outer 3/16 inch of its circumference.  1c. Replace funnel top. Avoid over-tightening which can damage the MF or cause leakage.	
	2. Deliver a small volume of sterile buffered distilled rinse water inside the funnel.	2a. Use approximately 10 ml of water. 2b. Observe funnel for leakage. If any, disassemble unit and repeat from Step 1 after inspecting base of funnel for possible debris or damage. Persistent leakage will necessitate maintenance or replacement of funnel unit.	
	3. Deliver standard sample volume to funnel by using a sterile graduate.	3a. Thoroughly shake sample bottle prior to filling graduate. A minimum requirement would be 25 complete up-and-down (or back-and-forth) movements of about 0.3 m (1 foot) in 7 seconds. 3b. Sterile graduate is prepared by oven sterilization with an aluminum foil cap.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)		3c. Volume in graduate is measured by sighting if the meniscus lower curve being even with the 100 ml mark.  meniscus line 100 90 eye level glass graduate (bottom of meniscus line touching 100 ml line in a parallel plane)	
	4. Gently pour standard sample (100 ml) into funnel.	4a. Avoid splashing. Pour slowly and close to top of funnel without touching sides. 4b. Allow a 5 second drainage period before shaking off the last drop.	
	5. Graduate marked TC (to contain): Rinse graduate several times with sterile water and pour each rinsing individually into funnel. Graduate marked TD (to deliver): Rinsing not necessary, but, allow at least 5 seconds drainage time and then gently tap off last drop into funnel.		

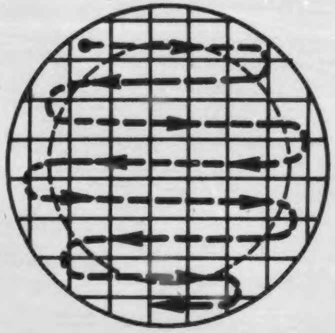
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)	6. Open vacuum control valve and remove pinch clamp to allow vacuum to filter sample through MF.	6a. Vacuum must not be allowed to enter system prior or during Step 5 as this would suck sample prematurely and bacterial dispersion will not occur over membrane. 6b. Allow complete passage of sample through MF.	
	7. Rinse funnel three times with sterile buffered distilled water.	7a. Rinsings remove all of residual sample droplets from sides of funnel. 7b. Allow complete flushing of each rinse through membrane before applying next rinse. 7c. Use about 25 ml for each rinse and pass around funnel to rinse complete circumference (circular motion of hand around funnel) of funnel. Do not touch inside area of funnel.	
	8. Replace pinch clamp on suction hose.	8a. Interrupts vacuum delivery to flask. 8b. Will not allow MF to be lifted from base without possible damage due to strong suction being continued. 8c. Some laboratories may elect to use control valve for this operation and not use pinch clamp.	
6. Membrane Plating	1. Disconnect funnel locking device and lift funnel from base to expose MF.	1a. Best to hold funnel in one hand while using forceps with other. Some laboratories may elect to either: * Use a germicidal cabinet to hold funnel * Use a funnel holding device But, in any event, DO NOT place funnel where it can become contaminated if it is to be used for another sample.	V.B.6.1 (p. 5-33)
	2. Remove membrane from funnel base.	2a. Again, handle membrane carefully with flamed forceps (quickly flamed after removing from alcohol immersion jar - NOT HEATED) and only on outer 3/16 inch of membrane.	

WATER MONITORING PROCEDURE: Total Coliform Test for Drinking Water by the Membrane Filter Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)		2b. Break residual vacuum in flask by gently lifting edge of MF before removing.	
	3. Replace funnel on base if it is held in other hand, or, replace when convenient if held in holding device or UV light box.	3a. Funnel unit is now ready to receive the next sample as the three rinses have been found to be sufficient to cleanse the funnel of bacteria which can influence this test.	V.B.6.3 (p. 5-34)
	4. Remove cover from m-ENDO dish. Do not allow it to become contaminated. Can either be held in the hand or placed on the lab bench.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)	5. Place MF over the m-ENDO medium. Use a rolling action to eliminate air pockets. Close the dish tightly when membrane shows elimination of air pockets.	5a. Grid or inked side surface contains the bacteria and must not be placed next to the m-ENDO. 5b. If air pockets persist (indicated by white areas with the pink colored m-ENDO liquid) pick up the MF by its edge and re-roll. Persistent clear or white areas usually indicate that there is too little broth on the pad. Add a drop of m-ENDO to the pad if necessary while holding up a corner of the MF. Do not place broth over the membrane. 5c. Do not run forceps or any object over the MF as it is very delicate and damage can result in poor plate results. 5d. Some amount of air spots is tolerable if they are outside the working area of where the bacteria were plated. About 3/16 inch is acceptable.	
7. Incubation Procedure	1. Invert petri dish (turn upside down). The bottom or plate base will now be on top and the MF will be upside down.		
	2. Place dish in the inverted position within the 35° C incubator.	2a. Plate is inverted to prevent droplets from "falling down" on MF destroying the colonial growth of the bacteria. 2b. Do not crowd plates. If a number of them have to be stacked, place them no more than three high with an unused area around them equal to the size of a petri dish. 2c. Allow an incubation period of 22-24 hours. Be sure time of plating is indicated on data sheet.	

WATER MONITORING PROCEDURE: Total Coliform Test for Drinking Water by the Membrane Filter Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second Day Procedure 1. Counting Procedure	1. Remove petri dish from incubator with careful handling to avoid jarring of plate. Turn plate over where cap is now on top.	1a. Incubation period has been within the 22-24 hour period. No deviations are permitted. 1b. Rough handling can cause spattering of droplets within plate and possibly causing difficulty in counting.	
	2. Remove cover to facilitate counting and place plate bottom on stage of microscope or under lens of magnifier.	2a. MF colonies are best counted with a magnification of 10 to 15 diameters. A wide field dissecting microscope is preferred but a magnifier is acceptable.	
	3. Adjust cool, white, fluorescent light source to give maximum sheen development to colonies, if any.	3a. A nearly vertical light adjustment is usually optimum. 3b. Do not use any other lighting source than the cool, white, fluorescent bulb. 3c. Sheen is characteristic of the coliform group of bacteria and is a golden, metallic-like reflective property on the surface of the colonies.	
	4. Microscopically scan membrane with a back-and-forth movement over the grids and count all colonies having sheen.	4a.  The dashed circle indicates the effective filtering area. The dashed back-and-forth line indicates the colony counting pathway.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second Day Procedure (Continued)		4b. Sheen development may be in a variety of forms - solid sheet, pin points, flecks, etc. Any amount of sheen is enough to consider the colony as being presumptively a coliform.	
		4c. Colonies are raised, usually circular, growths of original bacteria which was planted on the membrane and are considered to be the result of a single organism multiplying many times to produce a visible colony.	V.C.1.4c (p. 5-34)
	5. Observation of numerous colonies on the MF, even if they are not sheen containing colonies, will require counting since there is a 200 count maximum allowable colony count.	5a. The 200 colonies or more of all types (which includes coliforms) is an amount of growth which produces interferences with validity of results. 5b. A resampling should be requested in this case, and if persistent, treatment of the source must be instituted before laboratory results can be meaningful. It may be useful to run two 50 ml plates or four 25 ml plates and add the coliform colonies from the series to reduce the background colonies produced on the larger volumes. Thus, individual acceptable plates are obtained and the standard volume is still being processed.	
2. Recording Results	1. Since 100 ml is the standard sample volume, the number of coliforms counted will be the count/100 mls and this value is recorded on the data sheet.	1a. If any coliforms are present on the MF, each must be verified as being a coliform bacteria. 1b. In this case, close the dish and initiate the verification procedure as soon as possible. 1c. Turn in data sheet if no coliforms are present and take organizational policy steps if any coliforms are presumptively present.	V.C.2.1a (p. 5-34)

WATER MONITORING PROCEDURE: Total Coliform Test for Drinking Water by the Membrane Filter Method

SECTION	TOPIC
I	Introduction
II*	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI*	Field and Laboratory Reagents
VII	Field and Laboratory Analyses
VIII	Safety
IX	Records and Reports

Training guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

TRAINING GUIDE NOTE

REFERENCES/RESOURCES

A.10.2

Since 48 grams of m-ENDO broth powdered medium and 20 mls of 95% Ethanol are required to prepare 1 liter (1000 mls) of m-ENDO broth, it is possible to calculate weights and volumes to prepare any requirement based upon the number of plates desired. Calculations are based upon knowing the above figures and the requirement of 2.0 ml of broth for each pad saturation.

For rapid calculations the following two formulas can be used:

1. No. of plates desired x 0.096 = grams m-ENDO.
2. No. of plates desired x 0.04 = ml Ethanol.

EXAMPLE: If 47 plates of m-ENDO are required:

1. 47 x 0.096 = 4.512 or 4.5 grams m-ENDO.
2. 47 x 0.04 = 1.88 or 1.9 mls Ethanol.

NOTE: Due to the practical and technical difficulties involved in weighing very small portions as, for instance, 0.096 grams of m-ENDO for one plate requirement, it would be wise to prepare at least 10 plates (0.96 or 1.0 gram m-ENDO and 0.4 ml Ethanol) as a minimum requirement.

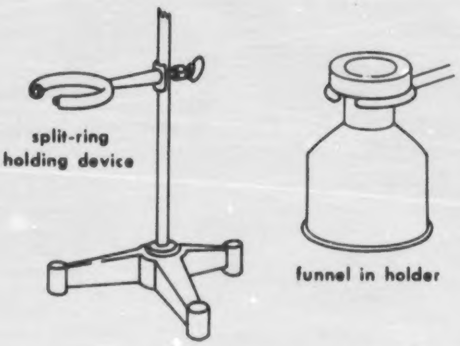
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.2b	A relative humidity of over 90 percent is necessary in order to obtain bacterial growth on the membrane filter (colony) which has not been inhibited by a lack of this moisture. Inhibited colonies will invariably be smaller and less apt to give the typical sheen characteristic of a frank coliform.	
	An accurate solid heat sink incubator is acceptable for use. This is constructed of a solid metallic block having slots for insertion of the petri dishes. Since there are no provisions for a high humidity chamber in this type of incubator, it is important to only use the types of petri dishes having a tight attachment of cover-to-base thus preventing loss of moisture during the incubation period.	Std. Meth. 14:937
	A closed container, such as a plastic vegetable crisper, may be placed within the incubator and have within the container a saturated humid atmosphere. A convenient way of accomplishing this is to wet a few paper towels and place within the crisper or box.	

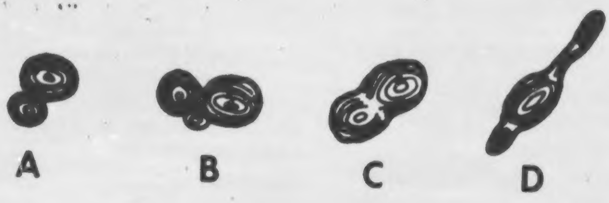
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1	Incubator should be kept out of drafts or direct sunlight in order to prevent temperature inside the incubator from changing outside the temperature range specified (35° C ± 0.5° C). Power supply should be selected so that there will not be too many pieces of equipment on the same circuit. Otherwise circuits will be blown repeatedly.	Standard Methods for the Examination of Water and Wastewater 14th Ed. (1975) APHA, WPCF, AWWA, p. 880 ff. (Hereafter referred to as: Std. Meth. 14:(Page No.)
A.1.3	Mercury bulb thermometer usually used in most incubators and a recording thermometer are acceptable. Thermometers must be calibrated against a mercury bulb thermometer which is (or calibrated against) a National Bureau of Standards issue and used with the certificate and correction chart.	
A.2	Sterilizing ovens should be of sufficient size to prevent crowding of materials to be sterilized. The information below summarizes the use of the oven.	Std. Meth. 14:881 :885

MATERIAL STERILIZED	CONDITIONS	REMARKS
Glassware	170° C for at least 60 min	If Internal oven characteristics are unknown
Glassware	160° C for at least 60 min	If oven temperature uniform throughout chamber
Glassware within metal container	170° C for at least 120 min	
Other material	170° C for at least 60 min	Material must be capable of withstanding sterilizing conditions

Alternately, a gas sterilizing unit can be used in place of the hot-air oven. Refer to Standard Methods and manufacturer's catalogs for details of such a unit (ethylene oxide gas).

Conflicting temperature/time relationships appear in differing references, but, the over-riding consideration is how this time/temperature relationship works in your hands, with your equipment, and considering the results of sterility testing.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.4	Distilled water must not contain substances preventing bacterial growth or be highly nutritive. There are required procedures for testing distilled water and should be undertaken only by professional bacteriologists or in laboratories where this is done regularly. Alternately, a source of deionized water which meets all requirements as imposed on distilled water is suitable for use in bacteriological operations.	Std. Meth. 14:887
B.4.2	Diagram and equipment listing describe the type of units most commonly used in the membrane filter procedure. They are by no means the only acceptable arrangement which can be utilized. Different modes of obtaining a vacuum or its control are available. Also, various vacuum flask arrangements are acceptable as well as the types of vacuum controls. To preclude numerous examples, the one described will be sufficient to give technological procedures which are acceptable and the reader can refer to Standard Methods and manufacturer's catalogs for further information regarding system components or field units which are acceptable.	
B.6.1	A germicidal cabinet is an enclosed unit which contains an active germicidal lamp (UV) which produces a 99.9% bacterial kill in 2 minutes. It is important not to have UV leakage from cabinet which can be detrimental to the analyst's eyes. A funnel holding device is designed to hold the funnel and prevent its contamination. EXAMPLE  	Std. Meth. 14:933

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.6.3	Funnel units are considered to be acceptable for use for the next sample unless an interval of 30 minutes or longer elapses before the next sample is run. In this case the unit must be resterilized.	Std. Meth. 14:932
C.1.4c	Occasionally colonial growth will be observed to be irregular such as the following:  Usually, as in A and B, the colonies are readily discernible as being multiple colonies - 2 for A and 3 for B. In the case of C and D, however, this separation is not readily apparent and the judgment, based on experience, of the analyst becomes important. In the case of D the long strand growth may be caused by a particle of debris which allowed channeled growth of one or more bacteria.	
C.2.1a	The verification test is accomplished by picking the presumptive sheened coliform colony with a sterile needle and passing it through a series of broth media to observe for another coliform characteristic-gas formation in a selective medium. Refer to Standard Methods for a detailed performance of this verification test.	Std. Meth. 14:920 :931

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

A.10.1 Procedures are given for m-ENDO broth medium preparation which is, however, not the only acceptable method available. Other acceptable m-ENDO medium preparations include:

A. m-ENDO Agar Medium

Std. Meth. 14:895

This includes the addition of the complex carbohydrate agar in order to solidify the medium. In this preparation the absorption pad is not required for analysis.

B. Pre-prepared Ampouled m-ENDO Medium

A complete prepared medium enclosed in a glass tube. Contains enough medium for a single medium for a single test and has the advantages of a larger shelf life and convenience of use. Is somewhat more costly than laboratory preparation.

A.10.4 Ethanol is added to distilled water in a 2% dilution for the m-ENDO medium. The amounts, of course, would be different depending on the petri dish requirements. The table below gives some useful information as reference:

No. of Plates m-ENDO Required	Alcohol-Water Required	Ethanol mls	m-ENDO Broth Powder gm.
500	1 liter (1000 ml)	20	48
250	500	10	24
50	100	2	4.8
25	50	1	2.4
10	20	0.4	.96 or 1.0
5	10	0.2	.48 or .5

Some laboratories prepare a large amount of the 2% solution and, when tightly stoppered, can be used for extended periods.

This outline was prepared by: Rocco Russomanno, Microbiologist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

VERIFIED MEMBRANE FILTER TEST FOR DRINKING WATER

as applied in

DRINKING WATER TREATMENT FACILITIES
and in the
DISTRIBUTION SYSTEMS OF DRINKING WATER TREATMENT FACILITIES

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

BA.MET.lab.WMP.8a.10.78

Verified MF Test for Drinking Water[1]

All typical coliform (sheen) colonies or at least five randomly selected sheen or borderline sheen colonies must be verified from each membrane containing five or more such colonies.

Needle must be sterile before selecting colonies.

Counts must be adjusted based on verification.

The verification procedure must be conducted by transferring growth from colonies into lauryl tryptose broth (LTB) tubes and then transferring growth from gas-positive LTB cultures to brilliant green lactose bile (BGLB) tubes. Colonies must not be transferred exclusively to BGLB because of the lower recovery of stressed coliforms in this more selective medium. However, colonies may be transferred to LTB and BGLB simultaneously. Negative LTB tubes must be reincubated a second day and confirmed if gas is produced.

It is desirable to verify all sheen and borderline sheen colonies (optional).

If there is more than one analyst in laboratory, at least once per month each analyst should count the sheen colonies on a membrane from a polluted water source. Colonies on the membrane should be verified and the analysts' counts compared to the verified count. (Optional)

Sheen colonies in mixed confluent growth reported and verified (optional).

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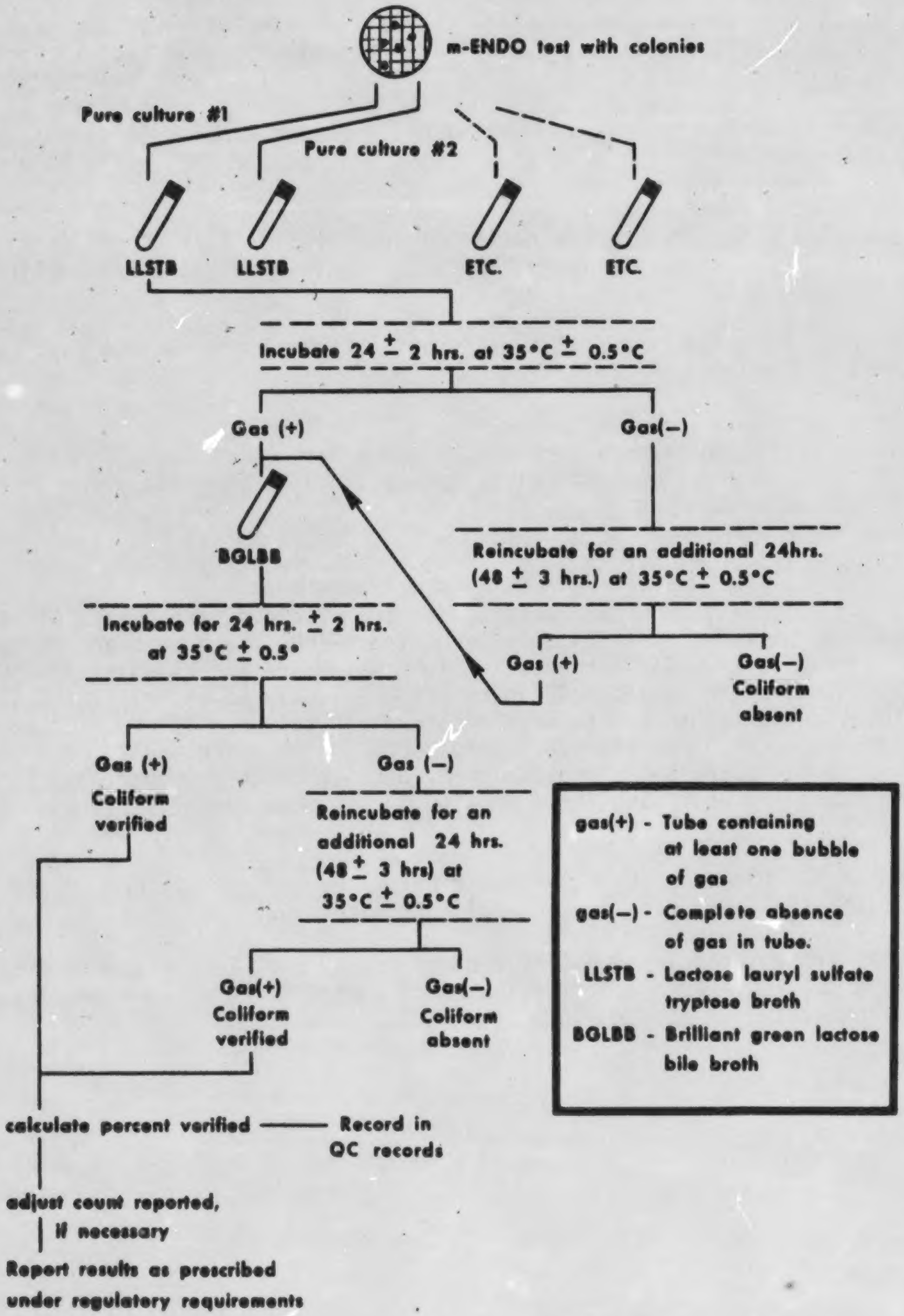
[1] MINIMUM REQUIREMENTS except where indicated as OPTIONAL.

Verified Membrane Filter Test Flow Sheet


m-ENDO test with colonies
Pure culture #1
Pure culture #2
LLSTB
LLSTB
ETC.
ETC.
Incubate 24 ± 2 hrs. at 35°C ± 0.5°C
Gas (+)
Gas(−)
BGLBB
Reincubate for an additional 24hrs. (48 ± 3 hrs.) at 35°C ± 0.5°C
Incubate for 24 hrs. ± 2 hrs. at 35°C ± 0.5°
Gas (+)
Gas(−) Coliform absent
Gas (+) Coliform verified
Gas (−)
Reincubate for an additional 24 hrs. (48 ± 3 hrs) at 35°C ± 0.5°C
Gas(+) Coliform verified
Gas(−) Coliform absent
calculate percent verified
Record in QC records
adjust count reported, if necessary
Report results as prescribed under regulatory requirements

gas(+) - Tube containing at least one bubble of gas

gas(−) - Complete absence of gas in tube.

LLSTB - Lactose lauryl sulfate tryptose broth

BGLBB - Brilliant green lactose bile broth

WATER MONITORING PROCEDURE: Verified Membrane Filter Test for Drinking Water

1. Analysis Objectives:

a. In water treatment plant quality control, the objective of the test is to determine if the effluent quality is in compliance with bacteriological requirements as prescribed in the Federal Drinking Water Standards.

b. In distribution network and individual consumer tapping locations, the test determines compliance with bacteriological requirements with the above mentioned standards.

c. Supplies conclusive determination of coliforms or lack of coliforms of mixed growth appearing in m-ENDO medium with apparent coliform-like colony(s) within mixed culture.

d. As part of a required Analytical Quality Control Program the test has applications for the following:

1) m-Endo medium quality check
2) laboratory personnel comparative check for new or established workers
3) supporting evidence of colony interpretations for legal or routine operations.

2. Brief Description of Analysis:

Discrete colony/s, whose characteristics are recorded, are aseptically (sterile technique) picked from a suitably incubated m-ENDO plate membrane filter and transferred to an LLSTB fermentation tube which are incubated at 35°C ± 0.5°C. After 24 hours and again at 48 hours (if previously negative), the LLSTB tube cultures are examined and results recorded for gas production. Cultures showing gas production are transferred to BGLBB fermentation tubes and incubated at 55° ± 0.5°C. BGLBB tubes are examined, usually at 24 hour periods, for gas up to 48 hours ± 3 hours and those showing gas are considered a positive coliform bacteria.

Based on the number of positives originally counted, it may be necessary to adjust the count reported for drinking water quality.

Results are entered in a quality control record book for necessary data pertaining to laboratory certification requirements or possible legal data requirements.

This procedure conforms to the Standard Total Coliform MPN Test as described in Standard Methods for the Examination of Water and Wastewater, 14th Edition (1975), p. 931.

WATER MONITORING PROCEDURE: Verified Membrane Filter Test for Drinking Water

Equipment and Supply Requirements

A. Capital Equipment:

Autoclave, providing uniform temperatures up to and including 121°C, equipped with an accurate thermometer, pressure gages, saturated steam power lines and capable of reaching required temperature within 30 minutes

Balance, 0.1 g sensitivity at load of 150 g

Microscope, stereoscope viewing, 5-15 x, fluorescent, vertical light source

Air incubator to operate at 35°C ± 0.5°C

pH meter, accurate to at least 0.1 pH unit, with standard pH reference solution(s)

Water distillation apparatus (glass or block tin), or source of distilled water suitable for bacteriological operations.

B. Reusable Supplies:

Apron or coat suitable for laboratory

Baskets, wire for discarded cultures

Bottle, squeeze type, with disinfecting solution

Burner, gas, Bunsen Burner type

Metal caps* to fit 25 x 150 mm and 18 x 150 mm culture tubes

Pan, to receive discarded contaminated glassware (must contain disinfectant before use)

Inoculation needle and loop, 3 mm diameter loop, of nichrome of platinum-iridium wire, 26 B&S gage, in holder

Racks, culture type*, having at least 5 openings capable of accepting tubes at least 20 mm in diameter

Sponge, for cleaning desk top

Tubes, culture*, 150 x 25 mm and 150 x 18 mm

Tubes, fermentation*, 75 x 10 mm vials to be inverted in culture tubes

C. Consumable (must be replaced when stocks get low):

Distilled water, suitable for bacteriological cultures (note distillation apparatus required in capital equipment)

Equipment and Supply Requirements (Continued)

BGLBB (Brilliant Green Lactose Bile Broth), dehydrated (recommend purchase of 1/4-lb. units)

LLSTB (Lactose Lauryl Sulfate Tryptose Broth), dehydrated (recommend purchase of 1-lb. units)

Disinfectant, for bench tops (Use household bleach solution prepared according to instructions on bottle.)

Wax pencils (recommend soft wax equivalent to Blaisdell 169T)

Data sheets (Verified Test)

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*Items marked are needed in quantities or require size or space allowances which cannot be specified here, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of "Standard Methods for the Examination of Water and Wastewater."

WATER MONITORING PROCEDURE: Verified Membrane Filter Test for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures		Aa. All pre-test procedures completed before starting other first-day procedures.	V.A.1 (p. 26)
1. 35°C Incubator Set-up, Adjustment	1. Place 35°C incubator in permanent location.	1a. Out of drafts or places where it will be in direct sunlight part of the day. 1b. Location convenient to laboratory bench. 1c. Convenient source of electric power.	V.A.1.2 (p. 26)
	2. Install thermometer.	2a. Thermometer functions at least in 30°-40°C range and have intervals of 0.5° or less indicated. Meets NBS standards. 2b. Location should be central in incubator. 2c. Mercury bulb thermometer should be fitted with cork or rubber stopper and mounted in small bottle filled with liquid (glycerine, water, or mineral oil).	V.A.1.2 (p. 26)
	3. Install shallow pan of water in bottom of incubator.	3a. In most laboratory incubators a pan having about 1 square foot area, with water about 1 inch deep, is satisfactory. 3b. Maintains condition of saturated relative humidity, required in bacteriological incubator. 3c. Requires daily check, with addition of water as necessary, to keep water in pan at all times.	V.A.1.3 (p. 26)
	4. Connect incubator to electric power source.	4a. Many incubators have pilot light to indicate power turned on.	
	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for method of temperature adjustment. 5b. Operation must be at 35 ± 0.5°C. 5c. Allow about 1 hour between adjustments.	V.A.1.5 (p. 26)
	6. Operate bacteriological incubator continuously.	6a. Requires daily check with written temperature record, with adjustment and water addition as necessary.	V.A.1.6 (p. 26)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)			
2. Autoclave Set-Up	1. Install and operate autoclave according to manufacturer's instructions.	1a. Autoclaves extremely variable in design and operation; also, potentially dangerous. 1b. Used to sterilize objects made of, or including liquids, rubber, culture media. 1c. Glassware may be autoclave sterilized but must be dried afterward. 1d. Most plastics not sterilized in autoclave; plastics usually require chemical sterilizers. 1e. Autoclave usually operated at 121°C for 15 min. 1f. Sterilized media must be removed from autoclave as soon as possible after autoclave is reopened.	V.A.2.1 (p. 26)
3. Water Distillation Equipment	1. Install and operate in accordance with manufacturer's instructions.	1a. Must produce distilled water meeting quality requirements for bacteriological tests	V.A.3.1-2 (p. 27)
	2. Operate continuously or intermittently as required to maintain adequate supplies of distilled water.	2a. Reserve supplies kept in borosilicate glass carboys or in plastic carboys made of material which will not dissolve substances which will affect growth of bacteria. 2b. Same distillation apparatus used for bacteriological purposes may be used for chemical reagents.	
4. pH Meter	1. Have unit available and operate in accordance with procedures described in other lab procedures.	1a. Unit for pH check on finished culture media. 1b. Used in preparation of stock solution of potassium dihydrogen phosphate.	V.A.4.1 (p. 28)
5. Glassware	1. Wash all glassware in hot detergent solution;	1a. Nontoxic detergent. 1b. Be sure all contents and markings are washed away.	V.A.5.1-4 (p. 28)
	2. Rinse at least once in hot tap water;		

WATER MONITORING PROCEDURE: Verified Membrane Filter Test for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	3. Rinse in distilled water, at least 6 successive times, and,		
	4. Dry in air.	4a. No visible spots or scum; glass should be clean, and sparkling. 4b. Glassware suitable for use in bacteriological operations.	V.A.5.4.4b (p. 28)
6. Preparation of Lactose Lauryl Sulfate Tryptose Fermentation Broth (LLSTB)	1. Weigh 17.8 grams of dehydrated Lactose Lauryl Sulfate Tryptose Broth. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media take moisture out of air; can become caked. 1b. Caked media unsatisfactory; should be discarded.	
	2. Dissolve in 500 mls distilled water.	2a. Gentle heat (no boiling) if necessary to complete dissolving medium. Usually a vigorous agitation will completely dissolve the medium.	
	3. Place 10.5 ml of the solution of prepared LLSTB in each culture tube.	3a. Use 150 x 18 mm tubes. 3b. 25 ml pipet, automatic pipetter, or funnel hose and pinchcock assembly are acceptable. 3c. Accuracy of delivery: ± 0.5 ml. 3d. Approximately 45 tubes will be necessary.	V.A.6.3b (p. 28)
	4. Insert one fermentation vial into each tube of medium, open end down.	4a. Tubes and vials washed as previously indicated. 4b. Use 75 x 10 mm tubes.	
	5. Place tube cap on each tube of culture medium.	5a. After all tubes have been filled and have individual vial.	
	6. Sterilize in autoclave.	6a. Within 1 hour after medium prepared. 6b. Sterilization at 121°C for 12 minutes. 6c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" mode of steam removal.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	7. Cool medium to room temperature.	7a. Medium ready for use when cool and individual vials are completely filled with fluid. No bubbles must be present.	
	8. Check pH of finished medium.	8a. Should be 6.7-6.9.	
	9. If final pH not satisfactory, discard medium and prepare new batch with pH adjustment before sterilization.	9a. pH value ordinarily drops about 0.2 pH unit.	
	10. Store medium in cool, dark place.	10a. Not in refrigerator. Usually in laboratory cabinet in darkness. 10b. May be stored up to 1 week if evaporation not more than 10% in loose fitting capped tubes. With screw-capped tubes, it should be held no longer than 3 months.	
7. Preparation of Brilliant Green Lactose Bile Broth (BGLBB)	1. Weigh 40.0 grams of dehydrated Brilliant Green Lactose Bile Broth. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media takes moisture out of air; can become caked. 1b. Caked media unsatisfactory; should be discarded.	
	2. Dissolve in 1 liter of distilled water.	2a. Gentle heat (no boiling) if necessary to complete dissolving medium. Usually a vigorous agitation will completely dissolve the medium.	
	3. Place 10.5 ml of the solution of prepared BGLBB in each culture tube.	3a. Use 150 x 18 mm tubes. 3b. A 25 ml pipet, automatic pipetter or funnel hose and pinchcock assembly are acceptable.	V.A.6.3b (p. 28)

WATER MONITORING PROCEDURE: Verified Membrane Filter Test for Drinking Water

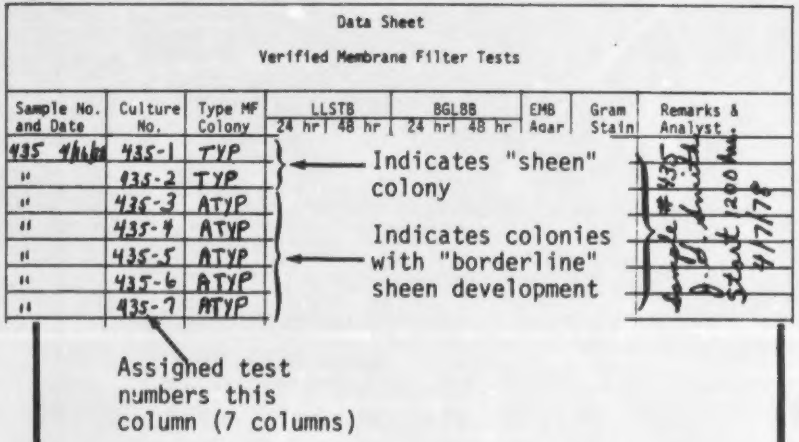
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedure (Continued)	4. Insert one fermentation vial into each tube of medium, open-end-down.	4a. Tubes and vials washed as previously indicated. 4b. Use 75 x 10 mm tubes.	
	5. Place cap on each tube of culture medium.	5a. After all tubes have been filled and have individual vial.	
	6. Sterilized in autoclave.	6a. Within 1 hour after medium prepared. 6b. Sterilization at 121°C for 12 minutes. 6c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" mode of steam removal.	
	7. Cool medium to room temperature.	7a. Medium ready for use when cool and individual vials are completely filled with fluid. No bubbles must be present.	
	8. Check pH of finished medium.	8a. Should be 7.1-7.3.	
	9. If final pH is not satisfactory, discard medium and prepare new batch with pH adjustment before sterilization.	9a. pH value ordinarily drops about 0.2 pH unit.	
	10. Store medium in cool, dark place.	10a. Not in refrigerator. Usually in laboratory cabinet in darkness. 10b. May be stored up to 1 week if evaporation not more than 10% in loose-fitting capped tubes. With screw-capped tubes, it should be held no longer than 3 months.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedure (Continued) 8. Final Equipment and Supply Check	1. Check to be sure that all equipment and supplies, solutions, and prepared media are ready before starting test procedures.	1a. Check general list of equipment and supplies. 1b. Each test requires: 1-5 tubes LLSTB 0-5 tubes of BGLBB 1 inoculation needle 1 inoculation loop 1 stereoscopic microscope (5-15x magnification) 1 burner 1 35° incubator Other: Sponge Disinfectant Wax pencil Data sheet Since, as shown, the numbers of items can vary (dependency upon the number of colonies picked from the m-ENDO plate for this procedure a hypothetical situation will be generated which will give the reader a cross section of conditions apt to occur.	
B. First-Day Procedures 1. Equipment Maintenance	1. Check, record and adjust incubator temperature. 2. Add water to pass in incubator as necessary.	1a. See previous information.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-Day Procedures (Continued)			
2. Unite Sample and Paperwork	1. Locate m-ENDO plate and corresponding data sheet of sample to be subjected to verification test.	1a. For purposes of this training exercise, our sample will be numbered "435" (data sheet and plate reflect this identification) and have the following characteristics: Data Sheet As required by agency or reporting facility m-ENDO Plate Count was made within 1 hour of initiating this procedure 7 colonies on filter: 2 frank sheen 5 borderline sheen	
		1b. Colonies to be subjected to verification test are usually predetermined during colony counting of m-ENDO plates since: A. All sheen colonies are tube verified, or, at least five sheen or borderline sheen colonies must be verified from each membrane containing five or more such colonies. B. It is optimally desirable to verify all sheen and borderline sheen colonies.	
3. Prepare Data Sheet for Test	1. Indicate on data sheet the required information.	1a. On "suggested" data sheet indicate: A. Sample number and date B. Culture number C. Type of Colony D. Analyst and start-of-test time. (See data sheet on following page.)	VII.B.3 (p. 29)

WATER MONITORING PROCEDURE: Verified Membrane Filter Test for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-Day Procedures (Continued)			



Data Sheet

Verified Membrane Filter Tests

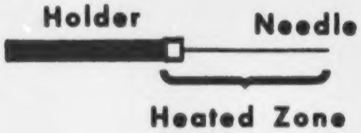
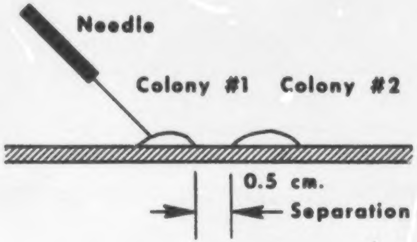
Sample No. and Date	Culture No.	Type MF Colony	LLSTB		BGLBB		EMB Agar	Gram Stain	Remarks & Analyst
			24 hr	48 hr	24 hr	48 hr			
435 4/6/78	435-1	TYP							Sample #435 D. [illegible] Start 1200 hrs 4/7/78
"	435-2	TYP							
"	435-3	ATYP							
"	435-4	ATYP							
"	435-5	ATYP							
"	435-6	ATYP							
"	435-7	ATYP							

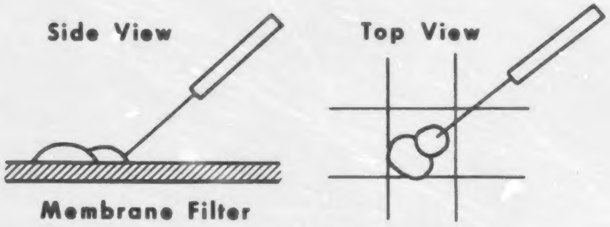
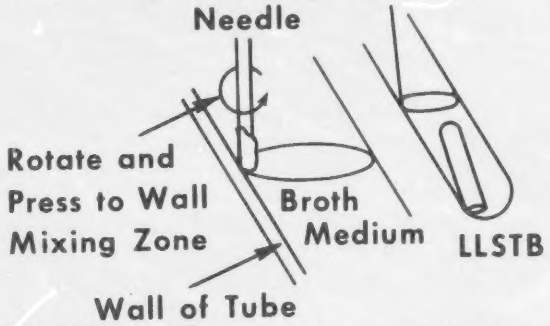
Indicates "sheen" colony

Indicates colonies with "borderline" sheen development

Assigned test numbers this column (7 columns)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Prepare Laboratory Test Area	1. Disinfect bench top; wipe dry.	1a. Sponge and disinfectant; paper toweling.	
	2. Assemble the following: 7 LLSTB tubes 1 Inoculation needle Sample 435 MF plate 1 Burner (light with match or striker) 1 Microscope with fluorescent light, 5-15x magnification		
5. Inoculation and Incubation of LLSTB Tubes	1. Starting with 435-1, which is a typical colony, focus at 15x the colony to be cultured.	1a. Remove cover from dish before placing it on microscope stage. 1b. Typical colony is characterized by metallic sheen. Any amount of sheen is considered positive.	III.B.5 (p. 25)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-Day Procedures (Continued)		1c. Have fluorescent light positioned vertically or near vertical to the plate. 1d. Focus microscope to give the sharpest image of the colony to be cultured.	
	2. Pick the colony with a sterile needle.	2a. Sterilize needle to red hot stage along the entire length of the needle in a burner flame. Allow flame to heat part of the holder for several seconds.  2b. Allow needle assembly to cool for about 5 seconds. 2c. With microscopic viewing, pick colony by allowing needle to penetrate growth mass. Withdraw needle from colony and pass away from scope. Do not touch needle at any time to anything but the colonial culture. 2d. Pick, if possible, only pure cultures for testing. A pure culture is one which shows a "separation" from the nearest neighboring colony of at least 0.5 cm: 	

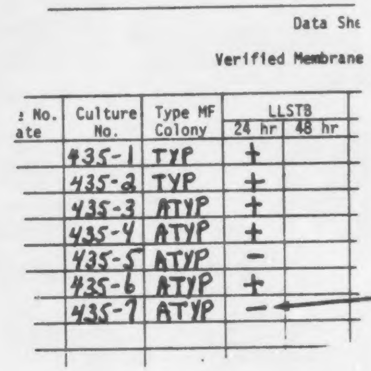
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-Day Procedures (Continued)		2e. If necessary to pick "touching" colonies, culture from the extreme end of the desired colony providing the greatest distance from the other colony:  	
		2f. If necessary to pick a sheen colony within a mass of background growth, simply touch the needle to the colony without regard to maintaining purity.	
	3. Transfer culture to LLSTB.	3a. Do not allow needle to touch anything as this could cause contamination to be transferred. 3b. Insert needle into LLSTB tube and "macerate" culture at glass-broth junction:  Needle Rotate and Press to Wall Mixing Zone Broth Medium LLSTB Wall of Tube 3c. Sterilize needle and return to storage location.	

WATER MONITORING PROCEDURE: Verified Membrane Filter Test for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-Day Procedures (Continued)	4. Identify LLSTB tube.	4a. Wax marking pencil: "435-1"	
	5. Repeat procedures for other colonies to be cultured.	5a. For 435-2; 435-3; 435-4; 435-5; 435-6; and 435-7.	
	6. Incubate all tubes at 35°C ± 0.5°C.	6a. For 24 hours ± 2 hours before inspection.	
	7. Disinfect lab test area; wipe dry.	7a. Sponge and disinfectant; paper toweling.	
C. Second-Day Procedures 1. Pre-Test Preparations	1. Accomplish equipment maintenance.	1a. As previously described.	
	2. Complete bench disinfection.	2a. As previously described.	
	3. Assemble data sheet and test components.	3a. Data sheet for this procedure. 3b. Components include: (#435 cultures) 7 LLSTB tubes from 35°C incubator.	
2. Read and Record LLSTB Results	1. Inspect the seven LLSTB tubes for gas formation and record results.	1a. Gently shake rack of tubes to allow possible supersaturation of gases to exit from solution. 1b. After several minutes, inspect for gas: any amount of gas in inner vial is considered positive.	

WATER MONITORING PROCEDURE: Verified Membrane Filter Test for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedures (Continued)		1c. Assume the following results:	



Data She

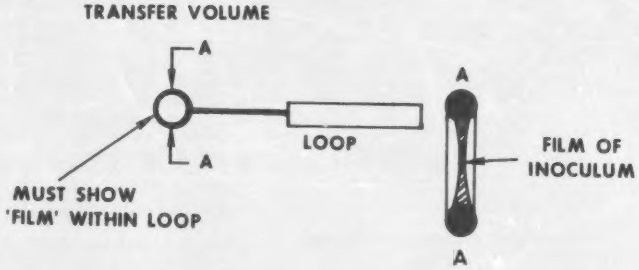
Verified Membrane

No. ate	Culture No.	Type MF Colony	LLSTB 24 hr	LLSTB 48 hr
	435-1	TYP	+	
	435-2	TYP	+	
	435-3	ATYP	+	
	435-4	ATYP	+	
	435-5	ATYP	−	
	435-6	ATYP	+	
	435-7	ATYP	−	

Enter results in this column

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
3. Prepare for and Complete BGLBB Transfers	1. Assemble LLSTB tubes for transfers.	1a. Five tubes will be required for transfers (all of the + tubes); two tubes will be re-incubated as they are negatives (435-5 and 435-7).	See Flow Sheet of Test Procedures
	2. Assemble materials required for transfers.	2a. Required: 1 Bacteriological loop 1 Burner 5 BGLBB sterile tubes Matches or striker Wax pencil	V.C.3.2 (p. 28)
	3. Transfer one loopful of culture from each LLSTB tube to a BGLBB tube.	3a. Only a sterile loop must be used to obtain culture from LLSTB tube. Flamed (to redness) and air-cooled loop must have film of inoculation for proper transfer: (See drawing on next page.)	

WATER MONITORING PROCEDURE: Verified Membrane Filter Test for Drinking Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedure (Continued)		 TRANSFER VOLUME A A LOOP MUST SHOW 'FILM' WITHIN LOOP A FILM OF INOCULUM A 3b. Transfer all positive LLSTB cultures to BGLBB tubes. Treat each tube aseptically (sterile technique) to avoid cross-contamination.	
	4. Label each BGLBB tube, then incubate five cultures.	4a. Wax pencil used for marking. 4b. Label each BGLBB tube to correspond to the LLSTB tube from which culture was obtained. 4c. Incubation at 35°C ± 0.5°C for 24 hours (first inspection).	
	5. Disinfect lab test area; wipe dry.	5a. Sponge and disinfectant; paper toweling.	
D. Third-Day Procedures 1. Pre-Test Preparations	1. Accomplish equipment maintenance.	1a. As previously described.	
	2. Complete bench disinfection.	2a. As previously described.	
	3. Assemble data sheet and test components.	3a. Data sheet for this procedure (#435 cultures). 3b. Components included: 2 LLSTB cultures (48 ± 3 hours incubation) 5 BGLBB cultures (24 ± 2 hours incubation)	

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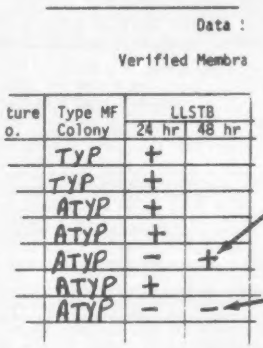
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedures (Continued) 2. Read and Record LLSTB Results	1. Inspect the 2 LLSTB cultures for gas production and record results.	1a. Gently shake rack of tubes to allow possible super-saturation of gases to exit from solution. 1b. After several minutes, inspect for gas; any amount of gas in inner vial is considered positive. 1c. Assume the following:  Culture 435-5 + in LLSTB... must be transferred to BGLBB Culture 435-7 - in LLSTB... coliform negative; discard tube	
3. Perform Transfer to Confirming Medium	1. Transfer culture 435-5 (LLSTB tube) to BGLBB.	1a. Loop transfer method as described previously. 1b. Label inoculated BGLBB tube with wax pencil as 435-5. 1c. Incubate this tube at 35°C ± 0.5°C.	
4. Read and Record BGLBB Results	1. Inspect the 5 BGLBB tubes for gas formation, process tubes, and record results.	1a. All tubes have been incubated for 24 ± 2 hours. 1b. Gently shake rack of tubes to allow possible super-saturation of gases to exit from solution. 1c. After several minutes, inspect tubes for gas; any amount of gas in inner vial is considered positive.	

Data :

Verified Membra

ture o.	Type MF Colony	LLSTB	
		24 hr	48 hr
	TYP	+	
	TYP	+	
	ATYP	+	
	ATYP	+	
	ATYP	−	+
	ATYP	+	
	ATYP	−	−

WATER MONITORING PROCEDURE: Verified Membrane Filter Test for Drinking Water

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>D. Third-Day Procedures (Continued)</td><td></td><td>

1d. Assume, for our continuing example, the following results:

Verified Mem

<table>
<tr><th>Culture No.</th><th>Type MF Colony</th><th colspan="2">LLSTB</th><th colspan="2">BGLBB</th></tr>
<tr><th></th><th></th><th>24 hr</th><th>48 hr</th><th>24 hr</th><th>48</th></tr>
<tr><td>435-1</td><td>TYP</td><td>+</td><td></td><td>+</td><td></td></tr>
<tr><td>435-2</td><td>TYP</td><td>+</td><td></td><td>+</td><td></td></tr>
<tr><td>435-3</td><td>ATYP</td><td>+</td><td></td><td>+</td><td></td></tr>
<tr><td>435-4</td><td>ATYP</td><td>+</td><td></td><td>+</td><td></td></tr>
<tr><td>435-5</td><td>ATYP</td><td>−</td><td>+</td><td></td><td></td></tr>
<tr><td>435-6</td><td>ATYP</td><td>+</td><td></td><td>−</td><td></td></tr>
<tr><td>435-7</td><td>ATYP</td><td>−</td><td>−</td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

+ BGLBB; verified for coliforms--discard tubes, unless further required

- BGLBB; must be re-incubated

1e. Incubate culture 435-6 at 35°C ± 0.5°C.
Note to summarize:

We have processed 7 cultures and at the present state we have the following status:

Verified as Coliforms
435-1; 435-2; 435-3; 435-4

Verification Negative (Not Coliforms)
435-7

Testing Incomplete (Pending)
435-5 (BGLBB tube in process)
435-6 (BGLBB still incubating)

</td><td>See Flow Sheet

III.D.4.1 (p. 25)</td></tr>
<tr><td></td><td>2. Disinfect lab test area; wipe dry.</td><td>2a. As described previously.</td><td></td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Fourth-Day Procedures 1. Continue Testing Procedure	1. Continue test procedures as previously outlined for 435-5 and 435-6.	1a. Assume the following results:	

Verified Membrane Filter Tes

Culture No.	Type MF Colony	LLSTB		BGLBB	
		24 hr	48 hr	24 hr	48 hr
435-1	TYP	+		+	
435-2	TYP	+		+	
435-3	ATYP	+		+	
435-4	ATYP	+		+	
435-5	ATYP	–	+	+	
435-6	ATYP	+		–	–
435-7	ATYP	–	–		

*BGLBB +; coliform verified

BGLBB -; coliform absent

*Note that this particular result could have gone to a fifth day of inspection had it been negative today.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
2. Analyze Verification Test Results	1. Calculate the percent verified for the sample.	1a. From the data sheet: 5 of 7 were + for coliforms; therefore: $\frac{5}{7} \times 100 = 71.4\%$	
	2. Record this percent verified on the data sheet.	2a.	

Remarks & Analyst
Sample #435
J. J. Smith
Sent 1200 hours
4/17/76
71.4% verified

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr>
<td>E. Fourth-Day Procedures (Continued)
3. Perform Data Adjustments Necessary</td>
<td>1. Adjust total coliform counts of original sample.</td>
<td>1a. Examples:
Suppose sample 435 had 12 colonies which could have been reportable (combination of frank sheen and borderline sheen colonies).
71.4% of 12 = 8.56 or 9 coliforms reported value
The same rationale is followed for other hypothetical values such as given below:
<table>
<tr><th>Sample MF Colonies</th><th>Calculated Value</th><th>Reported Value</th></tr>
<tr><td>11</td><td>7.85</td><td>8</td></tr>
<tr><td>9</td><td>6.42</td><td>*7</td></tr>
<tr><td>7</td><td>4.99</td><td>5</td></tr>
</table>
*As a factor of safety, report the "higher" value of fractional portions. Note: This procedure is not spelled out in any document, it is author's opinion.</td>
<td></td>
</tr>
</table>

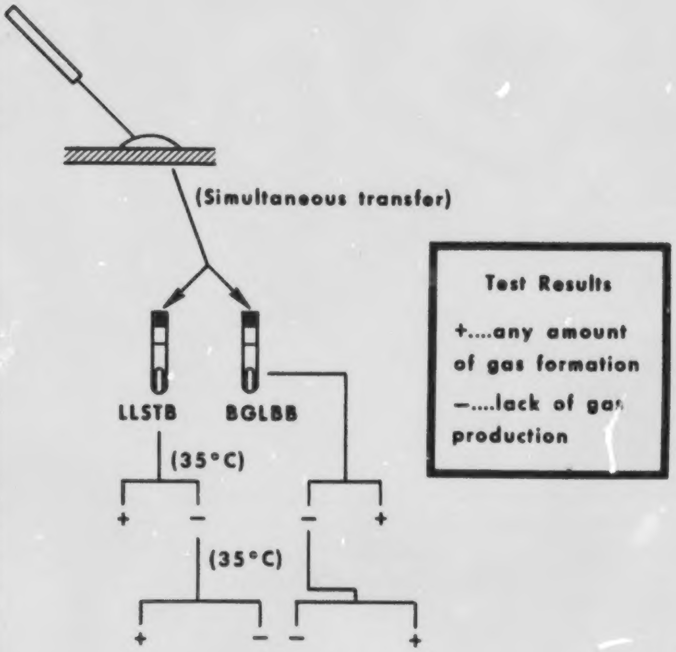
WATER MONITORING PROCEDURE: Verified Membrane Filter Test for Drinking Water

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

Training Guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.5	It is an acceptable procedure to also inoculate a BGLBB tube along with the LLSTB tube from the culture derived from the colony to be verified. This procedure can save from 1 to 4 days in the test procedure:	



Conclusions

Coliforms Present	Coliforms Absent	Remarks
LLSTB + and BGLBB +	LLSTB + and BGLBB - or LLSTB - and BGLBB -	Impossible to have LLSTB - and BGLBB +. If this occurs, inoculate the LLSTB with the culture from the BGLBB and observe for + within 48 hrs.

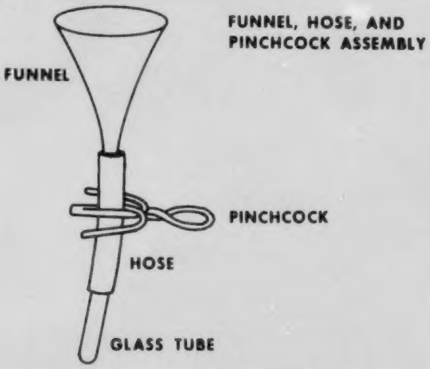
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
D.4.1	If requirements call for the continuation of testing to the gram staining procedure, it will be necessary to further process the BGLBB tube and not discard it at the positive stage.	Std. Meth. 14:918-19

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1	Incubator must be of sufficient size for daily workload without causing crowding of tubes to be incubated. Considerations for choice of incubator type must relate to reliability of operation and not to cost or attractiveness of equipment.	
A.1.1	Incubator should be kept out of drafts or direct sunlight in order to prevent temperature inside the incubator from changing outside the temperature range specified (35° ± 0.5°C).	Std. Meth. 14:880
	Power supply should be selected so that there won't be too many pieces of equipment on the same circuit. Otherwise, circuits will be blown repeatedly.	
A.1.2	Mercury bulb thermometer usually used in most incubators. Recording thermometer is acceptable, but, it should be calibrated against a mercury bulb thermometer which has been certified by National Bureau of Standards. The NBS certified thermometer always should be used with its certificate and correction chart.	
A.1.3	Saturated relative humidity is required in order to make the incubation more efficient (heat is transferred to cultures faster than in a dry incubator). Furthermore, culture medium may evaporate too fast in a dry incubator.	
A.1.5	Allow enough time after each readjustment to permit the incubator to stabilize before making a new adjustment. At least one hour is suggested.	
A.1.6	Incubator temperature can be held to much closer adjustment if operated continuously. Temperature records should be kept in some form of permanent record. A temperature record book is suggested with daily recording of values. If a recording thermometer is used, the charts may be kept as permanent record; if so, be sure that the charts are properly labeled to identify the incubator and the period covered.	
	Uniform temperature (35°C ± 0.5°C) is to be maintained on shelves in use.	
A.2.1	Autoclaves differ greatly in design and in method of operation. Some are almost like home-style pressure cookers; others are almost fully automatic. This is a subject which requires separate instruction; and should be related to the exact make and model of equipment you will use in your own laboratory.	Std. Meth. 14:881

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.2.1 (Cont'd.)	Vertical autoclaves and household pressure cookers may be used in emergency service if equipped with pressure gages and thermometers with bulbs positioned 1 inch above the water level. However, they are not to be considered the equivalent of the general purpose steam sterilizer recommended for permanent laboratory facilities. Their small size is inadequate for large-volume workloads, and they can be difficult to regulate. The following requirements must be met regarding autoclaves of sterilizing units: a. Reaches sterilization temperature (121°C), maintains 121°C during sterilization cycle, and requires no more than 45 minutes for complete cycle. b. Pressure and temperature gages on exhaust side and an operating safety valve. c. No air bubbles produced in fermentation vials during depressurization. d. Record maintained on time and temperature for each sterilization cycle.	
A.3.1-2	Distilled water in bacteriological laboratory must not contain substances which will prevent any bacteria from growing in culture medium in which the distilled water is used or will be highly nutritive. There are procedures for testing quality of distilled water; but these should be undertaken only by professional bacteriologists or in laboratories where this is done regularly. Use only glass stills or block tin lined stills.	Std. Meth. 14:645-49 14:888-89

Requirements for distilled water include the following:

Test	Analysis Requirement	Conducted
pH	4.5-8.5	Monthly
Conductivity	0.1 megohm as resistivity or <5.0 micromhos/cm at 25°C	Monthly
Trace metals:		
A single metal	Not greater than 0.5 mg/l	
Total metals	Equal or less than 1.0 mg/l	Annually
Test for bactericidal properties of distilled water (Std. Meth. 14:880)	0.8-3.0	Annually
Free chlorine residual	0.0	Monthly
Standard plate count	Less than 10,000/ml	Monthly

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.4.1	pH Meter: See cited reference.	Std. Meth. 14:882
A.5.1-4	Glassware: See cited reference on pipets and graduated cylinders, media utensils, bottles.	Std. Meth. 14:882-85
A.5.4.4b	Glassware can be checked for bacteriostatic or inhibitory residues by a bacteriological test procedure which, like the distilled water suitability test, should be undertaken only by professional bacteriologists or in laboratories where this test is done on a regular basis.	
A.6.3b	 FUNNEL, HOSE, AND PINCHCOCK ASSEMBLY FUNNEL PINCHCOCK HOSE GLASS TUBE NOTE: UNIT NEED NOT BE STERILE FOR MEDIUM DELIVERY ONLY.	
C.3.2	Alternately, it is authorized to use in "inoculation stick" for transfers and even for colony picking. A precisely sized and sterilized stick is intended for a one-time use and, if used, eliminates the need for a burner during the transfer or colony picking procedure. Of course, individual ones will be required for each tube as colony to be processed to maintain purity of culturing. Discard into a germicidal solution prior to discarding. Also available are re-sterilizable loops used once, re-sterilized, and available for future transfers.	Std. Meth. 14:917 14:883-84

B.3

There is no such thing as a "standard" data sheet for bacteriological tests. A suggested one is shown below and will be used for this training exercise:

Data Sheet

Verified Membrane Filter Tests

Sample No. and Date	Culture No.	Type MF Colony	LLSTB		BGLBB		EMB Agar	Gram Stain	Remarks & Analyst
			24 hr	48 hr	24 hr	48 hr			

Note: Some data sheets are made to include the verified test with the standard coliform MF test results on the same sheet. Whatever approach is made, however, it is important to have a place to indicate the reportable MF coliform count based on the results of the verified test which could cause a count adjustment.

This outline was prepared by: Rocco Russomanno, Microbiologist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinati, Ohio 45268

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

STANDARD PLATE COUNT

as applied in

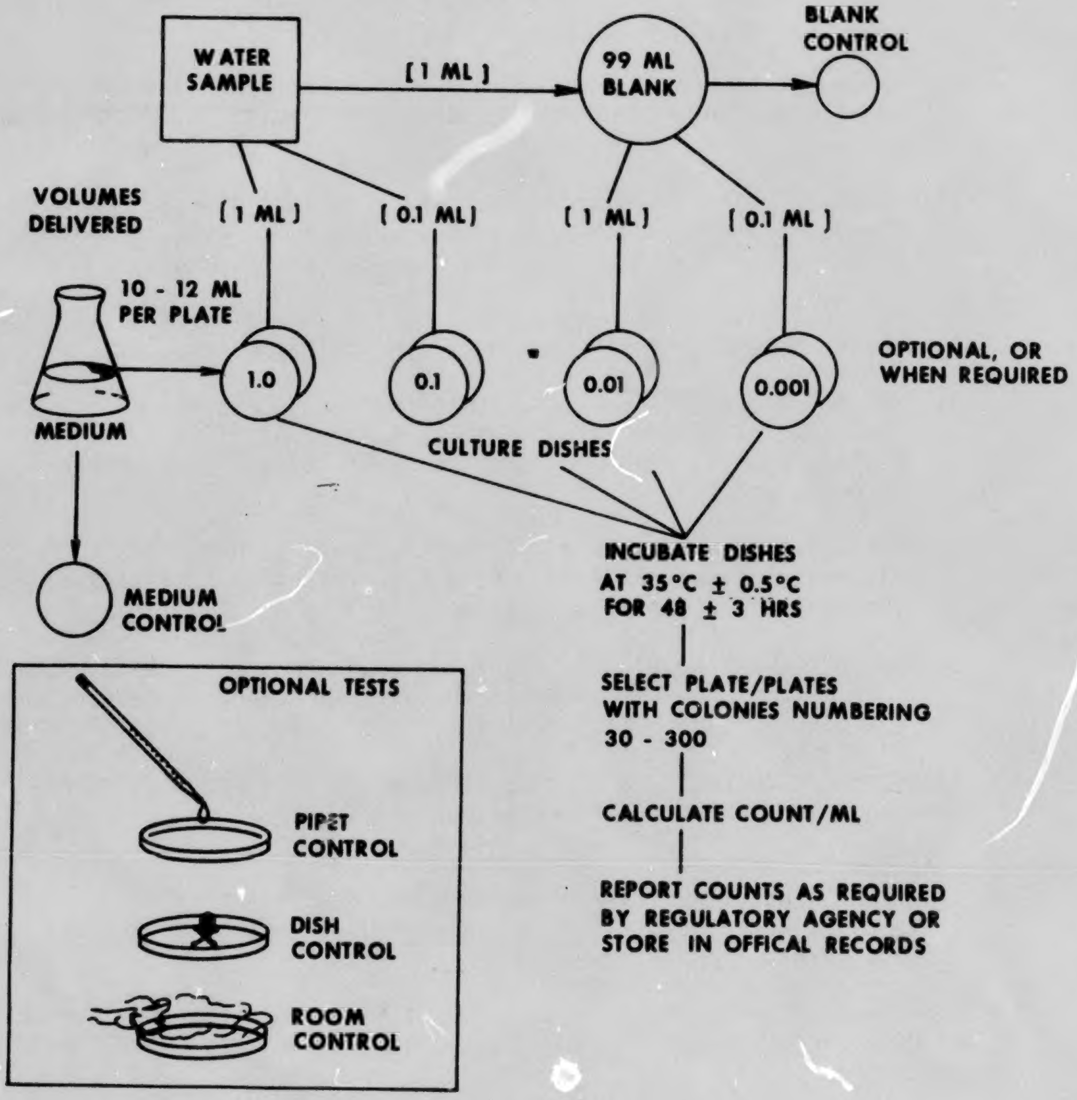
WATER TREATMENT FACILITIES
WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U. S. Environmental Protection Agency

BA.MET.lab.WMP.3.5.78

STANDARD PLATE COUNT

FLOW SHEET



WATER MONITORING PROCEDURE: Standard Plate Count

1. Applicability of Test:

   a. In water treatment plant, reservoir, and distribution line quality control, the objective of the test is to:

      1) provide a method of monitoring for changes in the bacteriological quality of finished water in storage reservoirs and distribution systems,

      2) indirectly limit the occurrence and magnitude of *Pseudomonas*, *Flavobacterium* and other secondary pathogenic invaders that could pose a health risk in the hospital environment,

      3) reduce problems in the detection of low densities of total coliforms due to interference by non-coliform bacteria,

      4) monitor the effectiveness of chlorine throughout the distribution network and provide a warning of filter effluent quality deterioration and the occurrence of coliform breakthrough, and

      5) indicate the existence of sediment accumulation in the distribution network that provides a protective habitat for the general bacterial population.

      Reference (1 - 5 above): Is the Total Count Necessary, Geldreich, E., AWWA Technology Conference Proceedings, Cincinnati, Ohio, December 3-4, 1973.

   b. Although not currently a test requirement, strong recommendations have been made for its future inclusion or use:

      Academy of Science
      National Interim Primary Drinking Water Regulations
      Office of Water Supply, U. S. Environmental Protection Agency
      (Dec. 1975).

   c. Knowledge of test procedure is required for conducting the "Suitability of Distilled Water Test" which is a required bacteriological test procedure.

   d. Establishment of "base line" general bacteriological data in conjunction with the coliform analysis regarding proposed regulations concerning modification of existing disinfection practices.

2. Brief Description of Analysis:

   A selected aliquot of water sample or its dilution is measured into a petri dish and a liquified, temperature controlled agar medium is added. An even distribution of organisms is accomplished by plate rotation and then the plate is allowed to harden prior to plate inversion and incubation at 35° ± 0.5° C for 48 ± 3 hours. Plate(s) having proper range(s) of colonies are counted and calculation(s) made to determine the count/ml.

3. Applicability of this Procedure:

   a. The range of total count concentrations:

If the sample volumes used are	These ranges of total count organisms covered are
1 ml, 0.1 ml and 0.01 ml	30 to 30,000/ml

   b. Pretreatment of samples in accordance with Standard Methods, 14th Ed. (p 904).

This procedure conforms to the Standard Plate Count as described in Standard Methods for the Examination of Water and Wastewater, 14th Ed. (1975), p. 908.

WATER MONITORING PROCEDURE: Standard Plate Count

Equipment and Supply Requirements

A. Capital Equipment:

1. Autoclave*, providing uniform temperatures up to and including 121° C, equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperature within 30 minutes.
2. Balance, 0.1 g sensitivity at load of 150 g.
3. Incubator*, air, to operate at 35° C ± 0.5° C.
4. Oven*, hot-air sterilizing, to give uniform temperatures and with suitable thermometer to register accurately in range of 160-180° C.
5. pH Meter, accurate to at least 0.1 pH unit, with standard pH reference solution(s).
6. Water Distillation Apparatus*, glass or block tin, or source of distilled water suitable for bacteriological operations.
7. Incubator*, water, to operate at 45° ± 1° C.
8. Refrigerator*, to operate at 4° C.
9. Thermometer, mercury bulb, certified NBS or calibrated against a certified NBS thermometer 0.5° intervals and have 160-180° C as part of range.

B. Reusable Supplies:

1. Apron or coat suitable for laboratory.
2. Baskets, wire for discarded cultures.
3. Bottles, dilution*, 6 oz. screw caps, with 99 ml volume level etched on one side.
4. Bottles, sample*, preferred characteristics being 250 ml (6-8 oz.), wide mouth, glass stopper.
5. Bottle, squeeze type, with disinfecting solution.
6. Burner, gas, Bunsen burner type.
7. Cans, pipet, aluminum or steel; not copper (If plastic, or other type of prepackaged disposable pipets are used, this item is unnecessary.)
8. Counter, colony, Quebec type, Darkfield model with guide plate, hand talley.
9. Cylinder, graduated, 100 ml.
10. Cylinder, graduated, 500 ml.
11. Dish*, petri, sterile, 100 mm diameter, ≥ 15 mm in height, with glass or porous tops preferred (presterilized, sterile one-time-use plastic tubes may be used).
12. Flask*, Erlenmeyer, 250 ml capacity.
13. Flask, Volumetric, 1 liter.
14. Pan, to receive discarded contaminated pipets and glassware (must contain disinfectant before use).
15. Pipets*, 1 ml, having 0.1 ml increments, sterile, cotton plugged, glass or disposable plastic, TD type (NOT a "blowout" type).
16. Pipets, 5 ml, having 1 ml increments (have several on hand).
17. Sponge, for cleaning desk top.
18. Thermometer, mercury bulb, certified NBS or calibrated against a certified NBS thermometer 0.5° intervals and have 30-40° C as part of range.

Equipment and Supply Requirements (Continued)

C. Supplies Used Up in the Analysis (must be replaced when stocks get low):

1. Cotton, nonabsorbent.
2. Disinfectant, for bench tops. (Use household bleach solution prepared according to instructions on bottle.)
3. Distilled water, suitable for bacteriological cultures (note distillation apparatus required in capital equipment).
4. EDTA (ethylene dinitrilotetraacetic acid).
5. Foil, aluminum.
6. Paper, Kraft.
7. Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$) (recommend purchase of 1/4 lb. units).
8. Pencil, wax, (recommend soft wax equivalent to Blaisdell 169T).
9. Potassium Dihydrogen Phosphate (KH_2PO_4) (recommend purchase of 1/4 lb. units).
10. Sheet, Data, SPC.
11. Sodium Hydroxide (NaOH).
12. Sodium Thiosulfate ($Na_2S_2O_3 . 5\ H_2O$).
13. Tryptose Glucose Yeast Agar, dehydrated medium (recommend purchase of 1/4 lb. unit).

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*Items marked are needed in quantities or require size or space allowances which cannot be specified here, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment of supplies, see the Microbiology Section of the current edition of Standard Methods for the Examination of Water and Wastewater.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures		Aa. All pretest procedures completed before starting other first-day procedures.	V.A.1 (p. 7-45)
1. 35° C incubator set-up, adjustment	1. Place 35° C incubator in permanent location.	1a. Out of drafts or places where it will be in direct sunlight part of day. 1b. Location convenient to laboratory bench. 1c. Convenient source of electric power.	V.A.1.1 (p. 7-45)
	2. Install thermometer.	2a. Thermometer functions at least in 30° - 40° C range and has intervals of 0.5° or less indicated. Meets NBS standards. 2b. Location should be central in incubator. 2c. Mercury bulb thermometer should be fitted with cork or rubber stopper and mounted in small bottle filled with liquid (glycerine, water, or mineral oil).	V.A.1.2 (p. 7-45)
	3. Install shallow pan of water in bottom of incubator.	3a. In most laboratory incubators a pan having about 1 square foot of area, with water about 1 inch deep, is satisfactory. 3b. Maintains condition of saturated relative humidity, required in bacteriological incubator. 3c. Requires daily check, with addition of water as necessary, to keep water in pan at all times.	V.A.1.3 (p. 7-45)
	4. Connect incubator to electric power source.	4a. Many incubators have pilot light to indicate power turned ON.	
	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for method of temperature adjustment. 5b. Operation must be at 35 ± 0.5° C. 5c. Allow about 1 hour between adjustments.	V.A.1.5 (p. 7-45)
	6. Operate bacteriological incubator continuously.	6a. Requires daily check with written temperature record, with adjustment and water addition as necessary.	V.A.1.6 (p. 7-45)

WATER MONITORING PROCEDURE: Standard Plate Count

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)			
2. Water bath incubator setup, adjustment	1. Place water bath incubator in permanent location.	1a. On bench or table surface. 1b. Out of drafts or place in which it will be in direct sunlight part of day. 1c. Location convenient to laboratory bench. 1d. Convenient source of electric power.	
	2. Put water in water bath	2a. Distilled or deionized water preferred, tap water accepted. 2b. Water must be deep enough that when a flask of medium is placed in the water bath the water is as high as the medium inside the flask. Yet it must not be so deep as to let the flask float or reach the cap or closure.	
	3. Install thermometer.	3a. Functions at least in 40° - 50° C range. Meets NBS standards. Have at least 0.5° C increment markings. 3b. Most water baths provide for corner location of thermometer (for protection from breakage).	
	4. Connect water bath incubator to electric power source and turn ON.	4a. Pilot light should come on.	
	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for location and method of temperature adjustment. 5b. Operation must be at 45° ± 1.0° C. 5c. Allow about 1 hour between adjustments.	
	6. Operate water bath incubator continuously.	6a. Requires daily check with written temperature record, with adjustment as necessary. 6b. Requires daily check of water level and addition of more as needed. 6c. With tap water in water bath, may require periodic scum removal from inner walls.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)			
3. Oven, sterilizer setup	1. Place oven sterilizer in permanent location.	1a. Convenient to source of electric power; usually on table or bench.	V.A.3.1-5 (p. 7-46)
	2. Install thermometer.	2a. Should indicate the 160° - 180° C range, be accurate within this interval, and be marked in 1.0 degree intervals. Thermometer bulb is within a cylinder filled with a fine sand and positioned on the center shelf of chamber.	
	3. Connect oven sterilizer to power source and turn on.	3a. Usually has pilot light to indicate power on.	
	4. Adjust temperature to stabilize at required temperature.	4a. Operated as near to 170° C as possible; not lower than 160 nor higher than 180° C. Check to verify that the 170° C temperature is reached and is maintained within ± 10° for a 2 hour period.	
	5. Operate oven sterilizer only when needed. Turn off when not in use.	5a. Turned ON in advance of need to permit reaching required temperature before introducing material to be sterilized. 5b. Oven sterilizer used to sterilize dry glassware, metal objects. 5c. Oven sterilizer NOT used with culture media, solution, plastics, rubber objects, or with anything containing or including these. 5d. Paper-wrapped glass pipets may be sterilized in oven sterilizer.	
4. Autoclave setup	1. Install and operate autoclave according to manufacturer's instructions.	1a. Autoclaves extremely variable in design and operation; also, potentially dangerous. 1b. Used to sterilize objects made of, or including liquids, rubber, culture media.	V.A.4.1 (p. 7-46)

WATER MONITORING PROCEDURE: Standard Plate Count

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)		1c. Glassware may be autoclave sterilized but must be dried afterward. 1d. Most plastics NOT sterilized in autoclave; plastics usually require chemical sterilizers. 1e. Autoclave usually operated at 121° C for 15 min. 1f. Sterilized media must be removed from autoclave as soon as possible after autoclave is reopened.	
5. Water distillation equipment setup	1. Install and operate in accordance with manufacturer's instructions.	1a. Must produce distilled water meeting quality requirements for bacteriological tests.	V.A.5.1-2 (p. 7-47)
	2. Operate continuously or intermittently as required to maintain adequate supplies of distilled water.	2a. Reserve supplies kept in borosilicate glass carboys or in plastic carboys made of material which will not dissolve substances which will affect growth of bacteria. 2b. Same distillation apparatus used for bacteriological purposes may be used for chemical reagents.	
6. pH meter operation	1. Have unit available and operational.	1a. Unit for pH check on finished culture media. 1b. Used in preparation of stock solution of potassium dihydrogen phosphate.	V.A.6.1 (p. 7-47)
7. Glassware preparation	1. Wash all glassware in hot detergent solution.	1a. Nontoxic detergent. 1b. Be sure all contents and markings are washed away.	V.A.7.1-4a (p. 7-47)
	2. Rinse at least once in hot tap water.		
	3. Rinse in distilled water, at least 6 successive times.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	4. Dry in air.	4a. No visible spots or scum; glass should be clean, and sparkling. 4b. Glassware suitable for use in bacteriological operations.	V.A.7.1.4b (p. 7-47)
8. Chemical solutions preparation for sample bottles	Sodium Thiosulfate		
	1. Weigh 10.0 grams of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$).	1a. Used for dechlorination of samples. Not required (but not detrimental to) for unchlorinated samples. 1b. Use of trip balance accepted.	
	2. Dissolve completely in 50-60 ml distilled water.	2a. 100 ml graduated cylinder satisfactory.	
	3. Add distilled water to bring final volume to 100 ml.		
	4. Transfer to labeled bottle.	4a. Labeled as 10% sodium thiosulfate; dated; preparer's name; and stored in refrigerator.	
	EDTA		
	5. Weigh 15.0 grams of EDTA.	5a. Used for water samples high in copper, zinc, or heavy metals. Normally not necessary for most treated water supplies. 5b. Use of trip balance accepted.	
	6. Dissolve completely in 50-60 ml distilled water.	6a. A 100 ml graduated cylinder is satisfactory.	
	7. Add distilled water to bring the final volume to 100 ml.		
	8. Transfer to labeled clean bottle.	8a. Labeled as 15% ethylene dinitrilotetraacetic acid (EDTA); dated; preparer's name; and stored in refrigerator.	

WATER MONITORING PROCEDURE: Standard Plate Count

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedure (Continued)			
9. Sample bottle preparation	1. Deliver 0.1 ml or .2 ml of 10% sodium thiosulfate solution to each sample bottle. (.1 ml to 4 ounce or 120 ml size and .2 ml to 6-8 ounce or 250 ml size).	1a. Use 1 ml pipet. 1b. Provides adequate sodium thiosulfate for neutralizing chlorine in sample. 1c. Return stock sodium thiosulfate solution to refrigerator.	V.A.9.1-6 (p. 7-48)
	2. Deliver .3 ml or .6 ml of 15% EDTA solution to each sample bottle (.3 ml to 4 ounce or 120 ml size and .6 ml to 6-8 ounce or 250 ml size).	2a. Use 1 ml pipet. 2b. Provides adequate EDTA chelating agent for metals in sample. Not necessary for sample which does not contain copper, zinc, or heavy metals. 2c. Return stock solution of EDTA to refrigerator.	
	3. Place cover on sample bottle.		
	4. Place paper or metal foil cover over bottle cap or stopper.	4a. Protects opening of sample bottle from accidental contamination.	
	5. Sterilize sample bottles in sterilizing oven.	5a. One hour at 170° C. (See A.3).	
	6. Store sample bottles in clean, dry place until used.		
10. Pipet preparation	1. Inspect pipets to be prepared for use; discard and destroy all having chipped or cracked tips or tops.	1a. Cleanliness of pipet must be equivalent to glassware.	

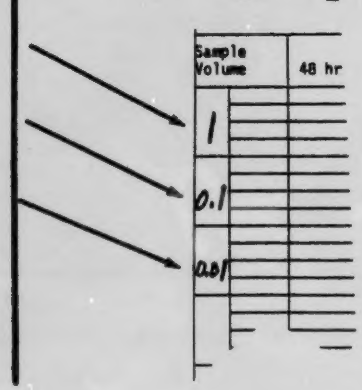
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedure (Continued)	2. Insert plug of non-absorbent cotton into mouthpiece of each clean, dry pipet.	2a. For protection of user when pipetting sample. 2b. Cotton plug must be tight enough to prevent easy removal, either by the pipetting action or by handling, and yet loose enough to permit easy air movement through the plug.	V.A.10.1-6 (p. 7-48)
	3. Place a layer of glass wool or several layers of paper padding in bottom of pipet can.	3a. For protection of pipet delivery tips within can.	
	4. Place 18-24 pipets in each pipet can, delivery tip down.	4a. Orientation permits removal of sterile pipets from can without contamination by user.	
	5. Sterilize cans of pipets in oven.	5a. 1 hour at 170° C. (See A.3 of procedures).	
	6. Store cans in clean, dry place until used. Mark cans as 1 ml sterile pipets.	6a. Laboratory cabinet or drawer recommended.	
	7. When can of pipets is opened for first use, pass the exposed ends of the pipets through flame, slowly.	7a. Burns off excess cotton sticking out of pipet mouthpiece. 7b. Cover kept on can at all times except when samples are being inoculated.	V.A.10.7 (p. 7-48)
11. Plate count agar preparation (tryptose glucose yeast agar)	1. Weigh 2.4 grams of dehydrated plate count agar. Cover bottle of medium tightly after removal.	1a. Dehydrated media can take moisture out of air (hygroscopic). 1b. Discard caked media; use only dry powder.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedure (Continued)	2. Dissolve in 100 ml of distilled water.	2a. Distilled water meets suitability requirements. 2b. Use 250 ml Erlenmeyer flask with foil cap. 2c. Use flowing steam (100° C) or boiling water to dissolve. Some autoclaves can be adjusted to give flowing steam mode. See manufacturer's instruction manual. 2d. Do not prolong boiling or exposure to steam. Agitate frequently when boiling is used to prevent burning. Boil for 1 minute. 2e. Expose to boiling or steam only until agar in medium has dissolved.	V.A.11.2b (p. 7-48)
	3. Close neck of flask with a plug of non-absorbent cotton. Cover with a cap of aluminum foil.	3a. Cotton tight enough to support weight of flask but not too tightly packed to resist easy removal.	V.A.11.2b (p. 7-48)
	4. Sterilize in autoclave.	4a. Within 1 hour after medium prepared. 4b. Sterilization at 121° C for 15 minutes. 4c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" (liquid cool) mode of steam removal. 4d. Total elapsed time from time of placement in autoclave to removal after sterilization, must not exceed 45 minutes.	
	5. Pour a small amount of medium (3-6 mls) into a small petri dish or clean container.	5a. Use sterile (aseptic) handling techniques to prevent medium contamination. Recap flask quickly.	
	6. Cool hot liquid medium to:		
	A. Room temperature and place in storage area.	6.A.a. If medium is to be used for future tests. 6.A.b. If more than three hours will elapse before test procedure.	
		6.A.c. Place in refrigerator (4°).	V.A.11.6.A.c (p. 7-49)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedure (Continued)	B. 44-46° C in water bath.	6.B.a. Hold for no longer than 3 hours. 6.B.b. If not used within the above time interval, cool to room temperature and place in refrigerator. (See A.11.6.A.c.)	
	7. Check pH of prepared medium in dish (See 11.5).	7a. Should be pH 7 ± 0.1. Probe(s) of pH meter can be inserted in medium. 7b. Record pH in Quality Control records. 7c. Discard plate after pH check. 7d. Medium will usually be within pH requirements. If not, reject prepared medium and check procedures, glassware, etc. for abnormalities. Prepare a new lot of Standard Plate Count medium when the cause has been found (glassware with acid residue, poor water supply, etc.).	
12. Dilution water blanks preparation	1. Prepare stock solution of potassium dihydrogen phosphate (KH_2PO_4); dissolve 34.0 grams of the KH_2PO_4 in 500 ml distilled water. Adjust to pH 7.2 with 1N NaOH, and dilute to 1 liter with distilled water.	1a. Distilled water may be measured in 500 ml graduated cylinder. 1b. Finished solution labeled "Stock KH_2PO_4 for Dilution Water." 1c. Stored in refrigerator. 1d. Discard stock solution and prepare new solution if mold appears.	V.A.12.1.1d (p. 7-49)
	2. Prepare stock solution of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) by dissolving 50 grams of this chemical in 500-600 mls of distilled water and, after complete dissolving, bring the final volume to 1 liter in a volumetric flask.		

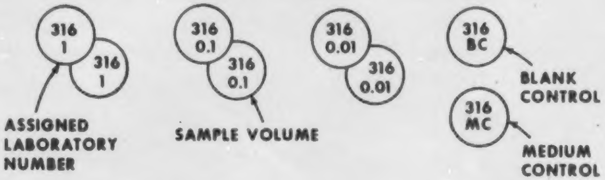
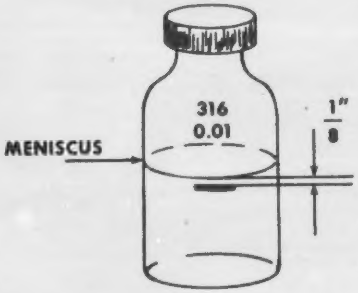
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedure (Continued)	3. Prepare working solution of dilution water by adding 1.25 ml KH_2PO_4 and 5 ml of the magnesium sulfate stock solution to each liter of distilled water to be made up as dilution water.	3a. 5 ml pipet satisfactory for 1 liter amounts of dilution water. 10 ml pipet better when several liters are being made. 3b. 1-liter graduated cylinder satisfactory for measurement of distilled water. 3c. Use separate pipets for each solution to prevent contamination.	
	4. Deliver enough working solution to each dilution water bottle so that after sterilization the bottles will contain 99 ± 2 ml of dilution water.	4a. 100 ml graduated cylinder ordinarily satisfactory. Pipetting machine desirable but not mandatory. Amount cannot be stated exactly, as sterilization evaporation differs from one autoclave to another. Commonly, about 102 mls are required.	V.A.12.4 (p. 7-49)
	5. Place caps on dilution bottles loosely.		V.A.12.5 (p. 7-49)
	6. Sterilize in autoclave.	6a. 15 minutes at 121° C. Use "slow-vent" mode of steam evacuation.	V.A.12.6 (p. 7-49)
	7. Promptly remove from autoclave, tighten bottle caps cool to room temperature.		
	8. Store in cool place.	8a. Dilution water ready for use. May be stored indefinitely in screw-capped bottles.	V.A.12.8 (p. 7-49)
13. Petri dish preparation	1. Clean and dry dishes; sterilize.	1a. If petri dishes are glassware, they meet requirements as previously described. Sterilize at 15/15 in the autoclave or 170° for 1 hr. in the oven. Glass dishes may be sterilized and stored in stainless steel or aluminum cans or wrapped in Kraft paper before sterilizing.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedure (Continued)		1b. If petri dishes are plastic and presterilized as purchased, they are used directly as taken from the packaging for single use only.	
14. Final equipment and supply check	1. Check readiness of equipment and supplies before starting sample examinations.	1a. Check general list of equipment and supplies.	
B. First Day Procedures			
1. Equipment maintenance	1. Check, record, and adjust incubator temperatures.		
2. Sample collection	1. Collect sample.	1a. Location as determined by requirement. 1b. Sampling methods as described in Standard Methods.	
	2. Record the on-site sampling informations.	2a. Most organizations have sample tags which at least include: Date of Sampling Time of Sampling Sample Locaton Collector's Name.	
	3. Transport sample to laboratory.	3a. Ideally under refrigeration (below 10° C) or in iced condition. 3b. If unrefrigerated, the maximum time allowable between collection and examination is 8 hours (NOTE: The maximum transit time is 6 hours). 3c. If extended holding time is unavoidable, maintain temperature below 10° C and do not exceed 30 hours between collection and examination.	

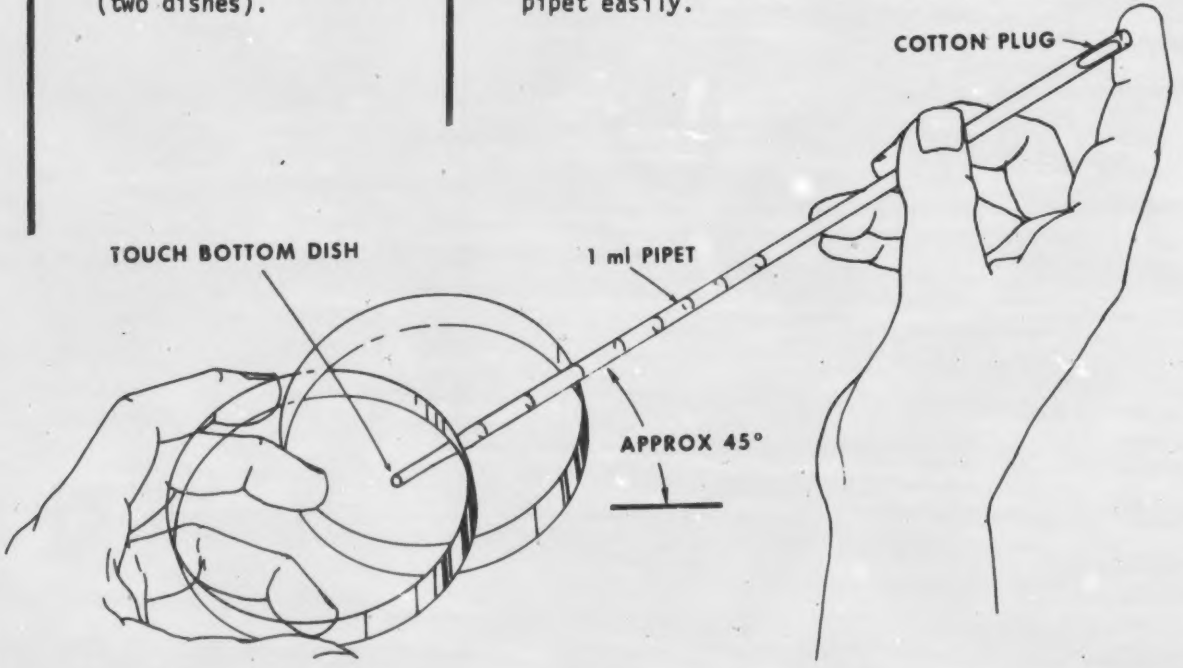
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedures (Continued)			
3. Preparation of data sheet	1. Fill in data sheet to show sample information.	1a. Required information should be on sample tag. 1b. Most data sheets record: Information as in B.2.2a Name of Analyst Laboratory sample Identification Time of Start of Analysis.	VII.B.3 (p. 7-53)
	2. Select sample inoculation volumes.	2a. For purposes of this WMP (Water Monitoring Procedure), sample volumes of 1.0 ml; 0.1 ml, and 0.01 ml are required. 2b. Above volumes are recommended for drinking water samples. 2c. Samples other than drinking water may require higher dilutions (Ex. 0.1; 0.01; 0.001).	
	3. Enter information in laboratory data sheet for sample volumes.	3a. Show sample inoculation volumes in milliliters (mls) or decimal amounts (1; 1/10; 1/100) (See VII.B.3).  	

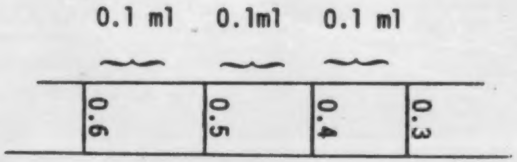
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedures (Continued)			
4. SPC medium preparation	1. Prepare or have available sufficient SPC medium. If medium is taken from refrigerator: (A.11.6.A)		
	A. Melt medium.	1.A.a. Melt by exposure to flowing steam or by placing flask in boiling water. 1.A.b. Heated only until fully liquified. DO NOT RESTERILIZE. 1.A.c. Only one remelting is authorized; discard if not used.	
	B. Cool to 44-46° C.	1.B.a. Place in water bath until at temperature (15 to 20 minutes for the 100 ml preparation). 1.B.b. Medium must not be held in incubator for over 3 hours.	
	If from water bath: (A.11.6.B)		
	C. Check that medium has not been incubated excessively.	1.C.a. Medium must not be held in incubator for over 3 hours.	
5. Laboratory bench disinfection	1. Disinfect laboratory bench; wipe dry.	1a. Sponge and disinfectant; paper toweling.	
6. Assembly of test related materials		B6. Consult general list of equipment and supplies.	

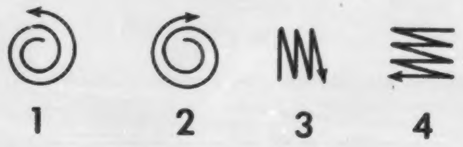
WATER MONITORING PROCEDURE: Standard Plate Count

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedures (Continued)			
	1. Label 8 sterile petri dishes for SPC Test.	1a. Three sample volumes run in duplicate. 1b. Label with wax pencil as follows:  316 1 316 1 316 0.1 316 0.1 316 0.01 316 0.01 316 BC 316 MC ASSIGNED LABORATORY NUMBER SAMPLE VOLUME BLANK CONTROL MEDIUM CONTROL	
		1c. Does not include "optional tests."	V.B.6.1c (p. 7-51)
	2. Select and label a a representative 99 ml dilution blank.	2a. Representative of a "batch" of sterilized bottles which were prepared and sterilized together. 2b. Select one which has meniscus above calibration mark, or, if all bottles are below mark: Transfer, by using aseptic (sterile) techniques, "batch" water from one bottle to another to above calibration mark (approx. 1/8 inch above). 2c. Label bottle with assigned sample number and 0.01 dilution volume:  	

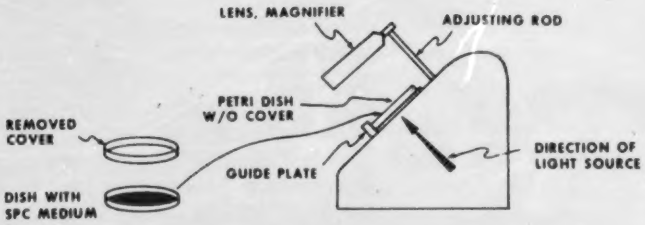
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedures (Continued)			
7. Sample inoculations, dilutions, and pouring	1. Agitate sample bottle.	1a. At least 25 shakes over space of at least 1 foot in 7 seconds or less.	I.B.7.1 (p. 7-40
	2. Fill 1 ml pipet with sample from sample bottle.	2a. Do not place pipet tip more than 1 inch below surface of sample. 2b. Do not wet cotton plug. 2c. Line sample water line with "0" marking on pipet. 2d. Touch off any droplets along inside of bottle before removing pipet. Do not touch outside of sample bottle. 2e. Close sample bottle; retain on bench for later use.	
	3. Place 1 ml volume in petri dishes labeled 1.0 ml (two dishes).	3a. Do not perform test in direct sunlight. 3b. Allow dishes to be opened only enough to insert pipet easily.	



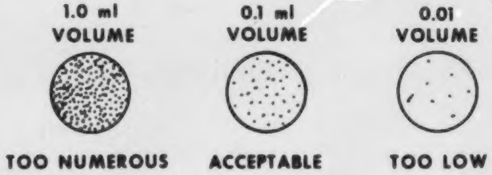
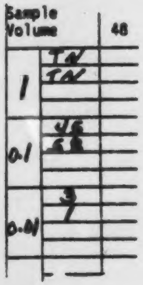
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedures (Continued)		3c. Pipet 1.0 ml volume into dishes. When volume has been delivered, touch off once any remaining droplet against a dry spot on the dish bottom and withdraw pipet. 3d. If pipet becomes contaminated touching bench; touching outside of petri dish; touching hand; etc.; discard and replace with a sterile one; repeat step. 3e. Close petri dishes immediately.	
	4. Place 0.1 ml volume in petri dishes labeled 0.1 ml (two dishes).	4a. Repeat steps 3b - 3e using the same sterile pipet but using a 0.1 ml volume and not retouching the plate. 4b. Any convenient 0.1 ml volume located between the 0 and 1.0 ml graduations is acceptable.  	
	5. Pour the four dishes (two 1.0 ml and two 0.1 ml).	5a. Use SPC medium from either B.4.1A-B or B.4.1C. 5b. Quickly pour 10-12 ml of the melted (44-46° C) SPC into each of the four dishes. 5c. Avoid splashing of medium. 5d. Open cover of dish only enough to allow ease of pouring of medium. 5e. Gently "swirl" each of the four dishes to obtain distribution of bacteria within the medium. Keep plates on bench while moving.	

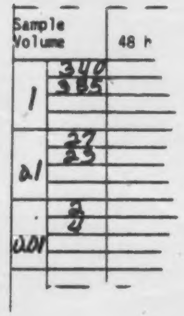
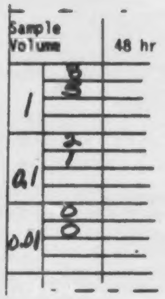
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedures (Continued)		5f. An acceptable "swirling" sequence could be as follows:  Do not allow motions to splash medium on cover of dish. If this occurs re-inoculate another dish. 5g. Allow plates to remain on bench to "harden" without disturbing.	III.B.7.5.5f (p. 7-42)
	6. Prepare blank control plate.	6a. Use previously labeled sterile petri dish "blank control" (316 BC). 6b. Using a sterile 1 ml pipet, remove water from the dilution blank after shaking as previously described, until the meniscus is even with the etched marking on the blank (See B.6.2.2a-c) and place this water into the blank control place. Close blank and retain for further requirement. 6c. Using techniques as in B.5.5a-g, prepare blank control plate.	
	7. Prepare 0.01 (or 10^{-2}) dilution.	7a. Shake sample bottle using agitation method previously described. 7b. Using a sterile 1 ml pipet, add 1 ml from the sample bottle to the 99 ml dilution blank which was prepared in B.6.6b. Discard pipet. 7c. Shake the dilution blank. Proper agitation previously described.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedures (Continued)	8. Place 0.01 ml volume in dishes labeled 0.01 ml (two dishes).	8a. Using a 1.0 ml delivery volume with a sterile pipet, follow the previous steps to place 1 ml volume in dishes from the dilution blank. Touch-off droplet against a dry spot. Close petri dishes. (Steps B.7.3.b-e).	
	9. Pour the two 0.01 dishes.	9a. Use previously learned steps for pouring (B.7.5.5.a-g).	
	10. Pour the MC (medium control) plate.	10a. Use previously labeled sterile dish (316 MC). 10b. Pour 10-12 ml of SPC medium into dish. Close dish immediately. 10c. Allow to harden. "Swirling" is not necessary as MC plate does not use sample.	
8. Incubation of plates	1. Collect all of the hardened plates.	1a. Eight plates should be ready for further processing (6 test plates; 2 control plates). 1b. Allow no more than 20 minutes to elapse from beginning test to collecting these plates. 1c. Hardened plates can be inverted (turned over) without flowing from fixed position.	
	2. Place inverted plates in 35° C incubator.	2a. Plates inverted to prevent condensation droplets from spreading on and causing irregular growth to occur on surface of medium. 2b. Do not crowd dishes in incubator. No more than four high and no touching of stacks with other stacks or top or sides of incubator. 2c. Incubate for 48 ± 3 hours.	

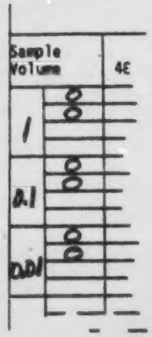
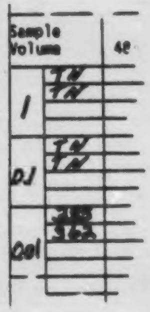
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedures			
1. SPC colony counting	1. Retrieve all plates from incubator (8).	1a. Bring to lab bench where colony counting is done. 1b. Assemble plates by sample volume 2 - 1 ml 2 - 0.1 ml 2 - 0.01 ml 1 - MC 1 - BC	
	2. Count control plates.	2a. Both MC and BC plates should show no bacterial growth. Occasional growth, such as less than 2 colonies, can be tolerated as chance contaminants.	III.C.1.2.2a (p. 7-42)
		2b. View plates with the Quebec Colony Counter. Good results are achieved (author method) by removing dish cover and placing dish on guide plate and then adjusting magnifier lens.  LENS, MAGNIFIER ADJUSTING ROD PETRI DISH W/O COVER REMOVED COVER DIRECTION OF LIGHT SOURCE GUIDE PLATE DISH WITH SPC MEDIUM	V.C.2.2b (p. 7-51)
		2c. If control plates are acceptable, proceed to the plate counts. If control plates are unacceptable, review procedures and/or discard contaminated materials and abort test until fresh materials are prepared for a fresh sampling.	

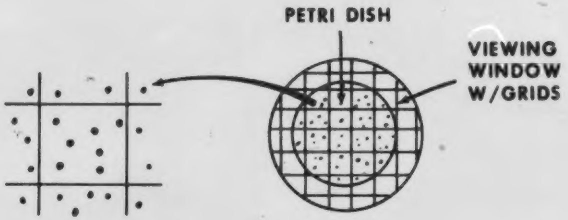
WATER MONITORING PROCEDURE: Standard Plate Count

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedures (Continued)	3. Count test plates.	3a. Scan the 3 pairs of plates for the ones which fall between 30-300 colonies/plate. 3b. With practice these can be easily ascertained, but, with the new worker counts would have to be made when uncertain until this skill is acquired. Example:  1.0 ml VOLUME 0.1 ml VOLUME 0.01 VOLUME TOO NUMEROUS ACCEPTABLE TOO LOW	
	4. Record counts on data sheet.	4a. Since a number of situations can be found related to counts, the possibilities are shown below: Plates having 30-300 colonies  Sample Volume 48 TN TN 1 0.1 3 7 0.01 NOTE: TN indicates Too Numerous (> 300) counts of 0.1 volume will be used for calculations.	

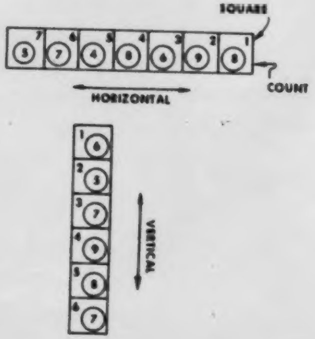
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedures (Continued)		4b. No plate with 30-300 colonies  Sample Volume 48 hr 1 340 355 0.1 27 23 0.01 3 2 NOTE: Counts of 0.1 volume will be used for calculations since they are CLOSER to the limits. 4c. All plates with fewer than 30 colonies  Sample Volume 48 hr 1 5 3 0.1 2 1 0.01 0 0 NOTE: Counts of 1 ml volume will be used for calculations since they are the LOWEST dilution plated (largest sample volume).	

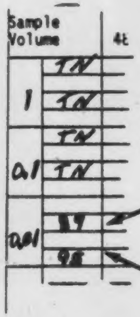
WATER MONITORING PROCEDURE: Standard Plate Count

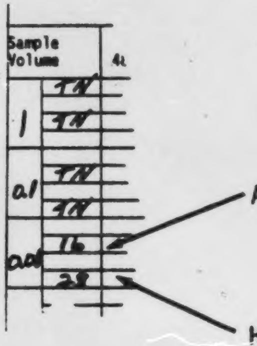
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedure (Continued)		4d. Plate with no colonies  Sample Volume 4e 1 0 0 0.1 0 0 0.01 0 0 NOTE: Counts of 1 ml volume (LARGEST VOLUME) will be used for calculations. 4e. All plates greater than 300 colonies  Sample Volume 4e 1 TN TN 0.1 TN TN 0.01 NOTE: Counts of 0.01 volume (SMALLEST VOLUME) will be used for calculations.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedure (Continued)		4f. Crowded plates using Quebec Counter to estimate Case #1: Less than 10 colonies per square cm A. There are less than 10 colonies per square cm when the number of colonies is less than 10 as the dish is viewed in the counter and one representative square is counted:  NOTE: 7 COLONIES IN CENTER SQUARE	

WATER MONITORING PROCEDURE: Standard Plate Count

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedure (Continued)		B. Count 13 squares which have representative colonial distribution. Seven (7) consecutive horizontal and six (6) consecutive vertical  SQUARE 7 6 5 4 3 2 1 5 7 4 8 6 9 8 COUNT HORIZONTAL 1 6 2 5 3 7 4 9 5 8 6 7 VERTICAL C. Sum Squares: 47 + 42 = 89 colonies. NOTE: When counting vertical and horizontal squares, do not count a square more than one time.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedure (Continued)		D. Assuming that the 0.01 ml dilutions had this situation, the entries would appear as follows:  Sample Volume 4t TN 1 TN TN 0.1 TN 0.01 98 As per example Hypothetically derived value from second plate using same rationale. Case #2 More than 10 colonies per square cm A. See C1.4.4f Case #1 for counting squares. B. Count 4 representative squares. For example: 12; 17; 13; 20. C. Average the count per sq. cm. 12 + 17 + 13 + 20 = 62 62 ÷ 4 = 15.5 or 16.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedure (Continued)		D. Assuming that the 0.01 ml dilutions had this situation, the entries would appear as follows:  Sample Volume 48 1 TN TN .1 TN TN .01 16 28 As per example Hypothetically derived value from second plate using same rationale.	
2. SPC calculations	1. Calculate count per ml.	1a. Plates having 30-300 colonies (See C.1.4.4a for example) Formula: $\frac{\text{Sum of colonies}}{\text{Sum of Volumes Tested, ml}} = \text{Count/ml}$ Given Example: $\frac{45 + 58}{.1 + .1} = \frac{103}{.2} = 515 \text{ Count/ml}$	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedure (Continued)		A special case exists when more than one dilution contains 30-300 colonies. Suppose, for example, that the following counts were recorded:	

Sample Volume	
1	TN TN
0.1	180 145
0.01	35 31

The following calculation is necessary:

$$\frac{180 + 145 + 35 + 31}{.1 + .1 + .01 + .01} = 1777 \text{ Count/ml}$$

1b. No plates with 30-300 colonies

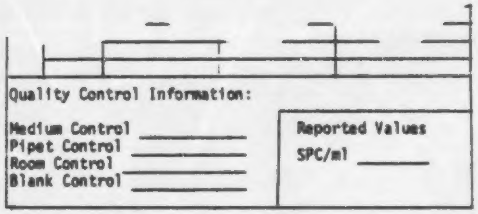
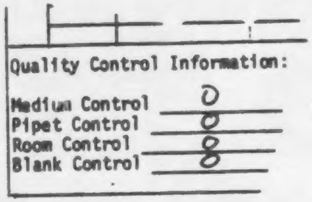
(See C.1.4.4b for example)

$$\frac{27 + 23}{.1 + .1} = \frac{50}{.2} = 250 \text{ Count/ml}$$

The counts of 27 and 23 were used since they were closer to the plate range of 30-300.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedure (Continued)		1c. All plates with fewer than 30 colonies (See example C.1.4.4c) $\frac{8 + 5}{1 + 1} = \frac{13}{2} =$ 6.5 or 7 Count/ml 1d. Plate with no colonies (See example C.1.4.4d) In order to use, for calculation purposes, a series of plates, none of which have colonies, assign a count of one (1) to each of the largest sample volume and calculate the count: $\frac{1 + 1}{1 + 1} = \frac{2}{2} =$ 1 Count/ml However, a count derived from this reasoning must be preceded by a less than (<) value. Therefore, the recorded count would be < 1 Count/ml. 1e. All plates greater than 300 colonies (See example C.1.4.4e) $\frac{385 + 360}{.01 + .01} = \frac{745}{.02} =$ 37,250 Count/ml	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedure (Continued)		1f. Crowded Plates	
		(See C.1.4.4f Case #1 and Case #2)	
		A. Case #1:	
		Multiply sum of 13 squares by 5:	
		Plate #1 89 (No. of colonies previously calculated) x 5 = 445.	
		Plate #2 95 x 5 = 475.	
		$\frac{445 + 475}{2}$ = 460 estimated count	
		B. Case #2:	
		Multiply the Count/sq cm (previously found to be 16 and 28) by 65 (No. of sq. cm. of petri dish):	
		Plate #1 16 x 65 = 1040	
		Plate #2 28 x 65 = 1820	
		Multiply by the reciprocal of the dilution to determine the count per ml.	
		Let us assume that the dilution was 0.01 ml for the plates being counted:	II.C.1.4.4f Case #2 (p. 7-41)
		Plate #1 1040 x 100 = 104,000	
		Plate #2 1820 x 100 = 182,000	
		$\frac{104,000 + 182,000}{2}$ = 143,000 Count/ml Estimated	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedure (Continued)	2. Record QC information and Count/ml on data sheet.	2a. See data sheet (VII.B.4):  Quality Control Information: Medium Control ______ Pipet Control ______ Room Control ______ Blank Control ______ Reported Values SPC/ml ______ 2b. With the usual conditions of good control and asceptic (sterile) handling techniques the QC information will be acceptable:  Quality Control Information: Medium Control 0 Pipet Control 0 Room Control 0 Blank Control 0	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Third Day Procedure (Continued)		2c. Record values to be reported: Reported Values SPC/ml ______	

For the examples given note the following:

Calculated Count per ml	Reported Count per ml (2 significant figures)
515	520 SPC (See C.2.1.1a)
1777	1800 SPC (See C.2.1.1a)
250	250 SPC (See C.2.1.1b)
7	7 SPC (See C.2.1.1c)
< 1	< 1 SPC (See C.2.1.1d)
37,250	37,000 SPC (See C.2.1.1e)
460	460 Estimated Plate Count (See C.2.1.1f Case #1)
143,000	140,000 Estimated Plate Count (See C.2.1.1f Case #2)

WATER MONITORING PROCEDURE: Standard Plate Count

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II*	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
VII*	Field & Laboratory Analyses
VIII	Safety
IX	Records and Reports

Training guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

1

INTRODUCTION

Section I

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.7.1	The SPC method for determining bacterial numbers are based on the assumption that the bacteria can be separated from one another (by shaking or other means) resulting in a suspension of individual bacterial cells, uniformly distributed through the original sample when the primary inoculation is made. SPC procedures are based on certain fundamental assumptions: A. First, all of the living bacterial organisms will remain viable if they are capable of growth under the conditions of the test; and; B. Second, resultant growth of the organisms will produce, within 48 hours at 35° C, a visible colony under appropriate magnification.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.1.4.4f Case #2	A reciprocal is the fractional "inversion" of a value (i.e., reciprocal of 4 is $\frac{1}{4}$; of $\frac{1}{4}$ is 4; etc.)	

Sample Volumes Used	Also Written As	Reciprocal
1.0	1	1
0.1	1/10	10
0.01	1/100	100

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

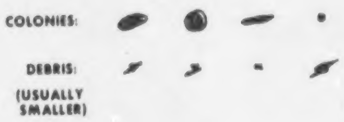
B.7.5.5f

A gentle "swirling" action allows the bacteria within the sample volume to mix with the SPC medium and be distributed evenly throughout the area of the petri dish bottom. Keeping the plate on the surface of the bench allows an easier management of the manipulation. Since the type of sample being considered in this WMP does not allow the novice to this procedure to "see" the sample being ultimately mixed and distributed, a "practice" plate can be made by using an opaque fluid (milk, cream, etc.) and seeing the gradual and complete mixing occurring as the sequential swirling is accomplished.

C.1.2.2a

Bacterial colonies are visible growths which have resulted from the multiplication of a simple organism which was trapped within the gelled agar-nutrient material. Colonial shapes are usually easily discernable forms which the beginner must learn to recognize and differentiate from debris which in this medium (SPC) is usually insoluble phosphate or undissolved particles.

Colonies are more uniform in shape than the debris particles:



A special type of colonial growth may sometimes be encountered which requires special counting rules. This growth is called a "Spreader" and constitutes an irregular and possibly extensive growth area which may or may not have originated from a single organism. Spreaders are usually top surface growth which therefore can grow with less restrictions than is the case within the agar mass where constraints to movement produce a small confined growth area. The presence of surface moisture as well as certain genera of bacteria can cause extensive growth areas.

C.1.2.2a
(Continued)

Plates containing spreading colonies must be so reported on the data sheet. If spreaders exceed one-half of the total plate area, the plate is not used. Report as: No results. Colonies can be counted on representative portions of plates if spreading colonies constitute less than one-half of the total plate area, and the colonies are well-distributed.

A. Count each chain of colonies as a single colony.

B. Count each spreader colony that develops as a film of growth between the agar and the petri dish bottom as one colony.

C. Count the growth that develops in a film of water at the edge or over the surface of the agar as one colony.

D. Report as: Estimated Standard Plate Count/ml.

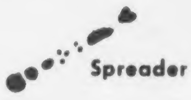
If spreading colonies (spreaders) are encountered on the plates/s selected, count colonies on representative portions only when

A. Colonies are well distributed in spreader-free areas, and

B. The area covered by the spreader/s does not exceed one-half the plate area.

When spreading colonies must be counted, count each unit of the following types as one:

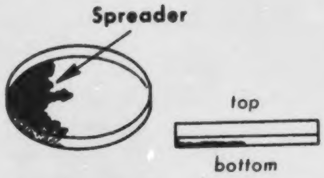
A. The first is a chain of colonies that appears to be caused by disintegration of a bacterial clump as the agar and sample were mixed. Count each such chain as a single colony, do not count each individual colony in the chain.



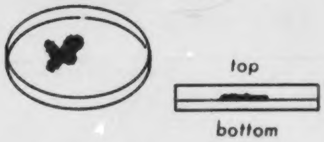
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

C.1.2.2a
(Continued)

B. The second type of spreader develops as a film of growth between the agar and the bottom of the petri dish.



C. The third type forms in a film of water at the edge or over the surface of the agar.



If plates prepared from the sample have excessive spreader growth, report as "Spreaders" (Spr). When plates are uncountable because of missed dilution, accidental dropping, and contamination, or the control plates indicate that the medium or other material or labware was contaminated, report as "Laboratory Accident" (LA).

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1	Incubator must be of sufficient size for daily work load without causing crowding of plates to be incubated. Considerations for choice of incubator type must relate to reliability of operation and not to cost or attractiveness of equipment.	
A.1.1	Incubator should be kept out of drafts or direct sunlight in order to prevent temperature inside the incubator from changing outside the temperature range specified (35° ± 0.5°). Power supply should be selected so that there will not be too many pieces of equipment on the same circuit. Otherwise, circuits will be blown repeatedly.	Standard Methods for the Examination of Water and Wastewater, 14th ed. 1975 APHA, WPCF, AWWA, p. 880 (Hereafter referred to as Std. Meth. 14: (page no.)
A.1.2	Mercury bulb thermometer usually used in most incubators. Recording thermometer is acceptable, but, it should be calibrated against a mercury bulb thermometer which has been certified by National Bureau of Standards. The NBS certified thermometer always should be used with its certificate and correction chart.	
A.1.3	Saturated relative humidity is required in order to make the incubation more efficient (heat is transferred to cultures faster than in a dry incubator). Furthermore, culture medium may evaporate too fast in a dry incubator.	
A.1.5	Allow enough time after each readjustment to permit the incubator to stabilize before making a new adjustment. At least one hour is suggested.	
A.1.6	Incubator temperature can be held to much closer adjustment if operated continuously. Temperature records should be kept in some form of permanent record. A temperature record book is suggested with daily recording of values. If a recording thermometer is used, the charts may be kept as permanent record; if so, be sure that the charts are properly labeled to identify the incubator and the period covered. Uniform temperature (35° C ± 0.5) is to be maintained on shelves in use.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.3.1-5	Since electric sterilizer will be operated intermittently, care should be taken that it is on a circuit which will not be overloaded when it is turned on. A time and temperature record is maintained for each sterilization cycle. Temperature recordings can be retained for records.	Std. Meth. 14:881
A.4.1	Autoclaves differ greatly in design and in method of operation. Some are almost like home-style pressure cookers; others are almost fully automatic. This is a subject which requires separate instruction; and should be related to the exact make and model of equipment you will use in your own laboratory. Vertical autoclaves and household pressure cookers may be used in emergency service if equipped with pressure gages and thermometers with bulbs positioned 1 inch above the water level. However, they are not to be considered the equivalent of the general purpose steam sterilizer recommended for permanent laboratory facilities. Their small size is inadequate for large-volume workloads, and they can be difficult to regulate. The following requirements must be met regarding autoclaves or sterilizing units: A. Reaches sterilization temperature (121° C), maintains 121° C during sterilization cycle, and requires no more than 45 min. for a complete cycle. B. Pressure and temperature gages on exhaust side and an operating safety valve. C. No air bubbles produced in fermentation vials during depressurization. D. Record maintained on time and temperature for each sterilization cycle.	Std. Meth. 14:881

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.5.1-2	Distilled water in a bacteriological laboratory must not contain substances which will prevent any bacteria from growing in culture medium in which the distilled water is used or will be highly nutritive. There are procedures for testing quality of distilled water; but these should be undertaken only by professional bacteriologists or in laboratories where this is done regularly. Use only glass stills or block tin lined stills. Requirements for distilled water include the following:	Std. Meth. 14:645-49 14:888-891

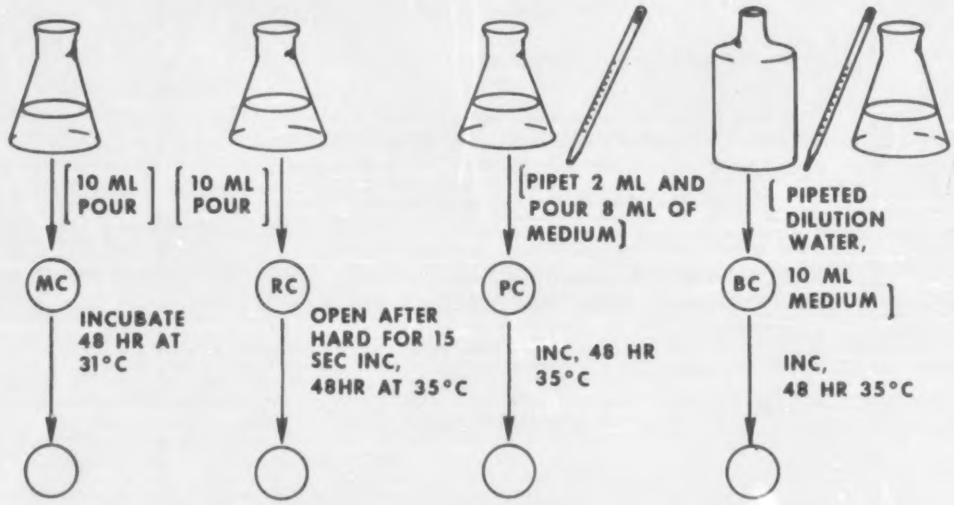
Test	Analysis Requirement	Conducted
pH	5.5 - 7.5	Monthly
Conductivity	0.1 megohm as resistivity or < 5.0 micromhos/cm at 25° C	Monthly
Trace Metals:		
A single metal	Not greater than 0.05 mg/l	Annually
Total metals	Equal to or less than 1.0 mg/l	
Test for bactericidal properties of distilled water ("Standard Methods," 14th Ed. p. 887)	0.8 - 3.0	Annually
Free chlorine residual	0.0	Monthly
Standard plate count	Less than 10,000/ml	Monthly

A.6.1	pH Meter: See cited reference	Std. Meth. 14:882
A.7.1-4a	Glassware: See cited reference on pipets and graduated cylinders, media utensils, bottles.	Std. Meth. 14:882-885
A.7.1-4b	Glassware can be checked for bacteriostatic or inhibitory residues by a bacteriological test procedure which, like the distilled water suitability test, should be undertaken only by professional bacteriologists or in laboratories where this test is done on a regular basis.	Std. Meth. 14:885

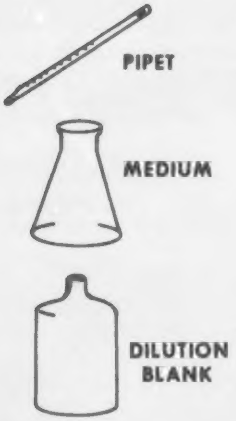
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.9.1-6	Sample bottles: Wide-mouthed glass-stoppered bottles suggested, but other styles acceptable. If glass-stoppered bottles are used, a strip of paper should be placed in the neck of the bottle before placing the stopper in place in preparation for sterilization. This prevents the glass stopper from "freezing" in place during sterilization. The paper strip is discarded at the time of sample collection.	Std. Meth. 14:884 14:904
A.10.1-6	Pipets: This procedure is described in terms of reusable glass pipets. However, single-service prepackaged glass or plastic pipets may be purchased and used, if preferred. In case of use of single-service pipets, they will be sterile when purchased, are used one time, and discarded immediately after use. Accordingly, in the step-by-step procedures disregard any instructions about preparation of pipets for reuse in case of using single-service pipets.	Std. Meth. 14:882-883
A.10.7	Passing the opened can of pipets through a flame burns off excess cotton wisps sticking out of the mouthpiece of the pipet. If this is not done, it is almost impossible to control sample measurement accurately. Some workers may elect to accomplish this step prior to the sterilization procedure.	
A.11.2b	Alternate medium containers which can be utilized are: 1. Flasks with screw caps 2. Tubes of at least 50 ml capacity with 15-20 ml of medium/tube 3. Dilution bottles It is preferable to use a container which has all of the medium for a single test since the medium control test will have greater test result assurance.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.11.6.A.c	Recommended Time Limits for Holding Prepared Media at 4° C Agar or broth in loose-cap tubes ..... One Week Agar or broth in screw-cap tubes, tightly closed .... Three Months Large volumes of Agar in screw-cap flasks or bottles, tightly closed .... Three Months	
A.12.1.1d	See cited reference. In time, this solution will become mold-infested. At this time it must be discarded and a new stock solution prepared.	Std. Meth. 14:892
A.12.4	Dilution water preparation: Measurement of dilution water into bottle with a 100 ml graduated cylinder is time-consuming, but effective. An automatic pipetting machine can be considered a luxury, but is a real time-saver.	
A.12.5	If caps are not placed on bottles of dilution water loosely, they may crack in autoclave; furthermore, steam will not be able to get in contact with the material being sterilized. After sterilization, tightening caps on bottles of distilled water will permit them to be kept for long periods.	
A.12.6	Always pack material loosely and away from walls in autoclave when preparing to sterilize. Steam must flow freely around materials being sterilized.	
A.12.8	If water should evaporate noticeably or become contaminated by microbial growth, the bottle of distilled water should be discarded.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.6.1c	Optional Tests are not run as routine procedures due to the fact that inordinate materials and time are required for their performance. They should, however, be occasionally run to establish that all materials and conditions are satisfactory. Possibly every 25th SPC test (author criteria) can be done in total and proper Quality Control records maintained to document satisfactory results. Indicated below are the full complement of control tests:	


10 ML POUR
10 ML POUR
PIPET 2 ML AND POUR 8 ML OF MEDIUM
PIPETED DILUTION WATER, 10 ML MEDIUM
MC
RC
PC
BC
INCUBATE 48 HR AT 31°C
OPEN AFTER HARD FOR 15 SEC INC, 48HR AT 35°C
INC, 48 HR 35°C
INC, 48 HR 35°C

OBSERVE PLATES (SHOULD NOT CONTAIN MORE THAN OCCASIONAL COLONIES OR < 3 COLONIES)



MC - MEDIUM CONTROL
RC - ROOM CONTROL
PC - PIPET CONTROL
BC - BLANK CONTROL

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.6.1c (Continued)	The majority of control tests will fall into the following patterns from which decisions can be made as to the status of materials:	

TEST RESULTS				REMARKS
MC	RC	PC	BC	
+	+	+	+	Medium possibly contaminated; petri dishes possibly contaminated; both of above possibly contaminated.
-	-	+	-	Pipet contaminated.
-	-	-	+	Blank contaminated.
-	+	-	-	Room atmosphere contaminated.

- .... No Growth, sterile plate
+ .... $\geq$ 3 colonies; indicates contamination

NOTE: Although 48 hours incubation time is stipulated, the plates should be examined at 24 hours since gross contamination can be observed at this interval and a + can therefore be found earlier.

C.2.2b

A number of alternately acceptable counters are available, and, if they can be shown to be equivalent to the discussed method of counting (manual), they would be acceptable for use.

Included among these counters are electronic-assist devices which registers each colony with a sensing probe and have an automatic tabulation. Recently a fully-automatic counter was made available which scans and registers all particles (colonies) above a preset threshold-size.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.3	There is no such thing as a "Standard" data sheet for bacteriological tests. Entries for the SPC may be an integral part of a multi purpose data sheet or be used only for the specific test. A simplified data sheet is presented below:	

STANDARD PLATE COUNT

Sample Type ____________________ Lab. No. _____

Station ____________ Description ____________

Collection Date ________ Time ____________ APM

Received ________ APM Examined __________ APM

Sampler Name ______________________________

Analyst Name ______________________________

Remarks __________________________________

Sample Volume		48 hr count	72 hr count	Count per ml

Quality Control Information:

Medium Control __________
Pipet Control __________
Room Control __________
Blank Control __________

Reported Values
SPC/ml _______

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	This outline was prepared by: Rocco Russomanno, Microbiologist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.	

RESIDUAL CHLORINE AND TURBIDITY

I. INTRODUCTION

The Interim Primary Drinking Water Regulations (Federal Register, December 24, 1975) permits the options of substitution of up to 75 percent of the bacteriological samples with residual chlorine determinations. Any community or non-community water system may avail themselves of this option with approval from the State based upon results of sanitary surveys. Residual chlorine determinations must be carried out at the frequency of at least four for each substituted microbiological sample.

Since many potable water plants carry out their own microbiological determinations, it will be necessary that these laboratories be certified for the bacteriological parameters. Residual chlorine determinations may be carried out by any person acceptable to the State and the analytical method and techniques used must be evaluated in some manner to assure that reliable information is obtained.

Since the presence of high turbidity can interfere with the disinfection capability of chlorine, a maximum allowable limit has been set for turbidity as follows:

A. One turbidity unit (TU) as determined by a monthly average except that five or fewer turbidity units may be allowed if the supplier of water can demonstrate to the State that the higher turbidity does not

   1. Interfere with disinfection,

   2. Prevent maintenance of residual of disinfectant throughout distribution system, or,

   3. Interfere with microbiological determinations.

B. Five turbidity units based on an average of two consecutive days.

The Criteria and Procedures Document for Water Supply Laboratory Certification suggests that some quality control guidelines be instituted for the residual chlorine and turbidity measurements at the State level for the purpose of ensuring data validity for these critical measurements.

In response to public comments regarding the proposed Primary Regulations (Federal Register, December 24, 1975) it is stated that operators performing residual chlorine and turbidity analyses "....be certified, approved, or at least minimally trained to perform the analytical tasks before a State could accept their analytical determinations...."

II. RESIDUAL CHLORINE

Since residual chlorine analysis would be carried out in "field" conditions or in the small laboratories of treatment plants, perhaps by unskilled operators, it is necessary to keep the analytical method as simple as possible. For a number of years, operators had utilized the orthotolidine technique in a kit form to determine the chlorine residual. Recent studies and regulatory guidelines have dictated against this test procedure. The acceptable test procedure is now the DPD Test (13th Ed., Standard Methods for the Examination of Water and Wastewater, pgs. 129-132), for which kits are available from at least two companies and which meet requirements for accuracy and reliability. These kits are capable of measuring both free and combined chlorine of which only the free chlorine is measured to meet compliance requirements. Kit procedures call for a premeasured single powder or tablet reagent added to the test cell with the sample and a resultant color development measures by comparison the standardized colors within one minute. Standard Methods includes cautions regarding temperature and pH control regarding this test parameter and this test procedure, the DPD Test, is least effected by temperature and the pH is adjusted by the added reagents. The only interfering substance, oxidized manganese, can be determined in a preliminary step and compensated for in the final test value.

III. TURBIDITY

Turbidity has long been used in the water supply industry for indicating proper operational techniques. Turbidity should be clearly understood to be an expression of the optical property of a sample which causes light to be scattered and absorbed rather than transmitted in straight lines through the sample.

The standard method for the determination of turbidity has been based on the Jackson candle turbidimeter. However, the lowest turbidity value which can be measured directly on the Jackson turbidimeter is 25 units which is well above the monitoring level. Because of these low level requirements, the nephelometric method was chosen and procedures are given in Standard Methods (13th Ed., 1971).

IV. NEPHELOMETRIC MEASUREMENTS FOR COMPLIANCE MONITORING

The subjectivity and apparatus deficiencies involved in visual methods of measuring turbidity make each unsuitable as a standard method.

Since turbidity is an expression of the optical property of scattering or absorbing light, it was natural that optical instruments with photometers would be developed for this measurement.

The type of equipment specified for compliance monitoring(3,6) utilizes nephelometry.

A. Basic Principle(7)

The intensity of light scattered by the sample is compared (under defined conditions) with the intensity of light scattered by a standard reference solution (formazin). The greater the intensity of scattered light, the greater the turbidity. Readings are made and reported in NTUs (Nephelometric Turbidity Units).

B. Schematic

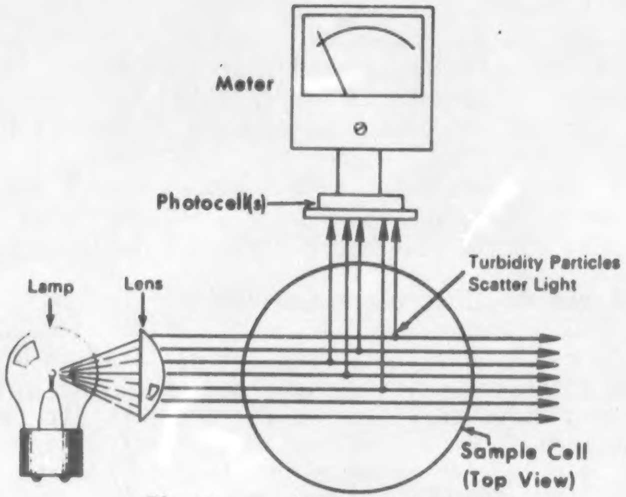


Figure 2 NEPHELOMETER (90° Scatter)

Light passes through a polarizing lens and on to the sample in a cell. Suspended particles (turbidity) in the sample scatter the light.

Photocell(s) detect light scattered by the particles at a 90° angle to the path of the incident light. This light energy is converted to an electric signal for the meter to measure.

1. Direction of Entry of Incident Light to Cell

   a. The lamp might be positioned as shown in the schematic so the beam enters a sample horizontally.

   b. Another instrument design has the light beam entering the sample (in a flat-bottom cell) in a vertical direction with the photocell positioned accordingly at a 90° angle to the path of incident light.

2. Number of Photocells

   The schematic shows the photocell(s) at one 90° angle to the path of the incident light. An instrument might utilize more than one photocell position, with each final position being at a 90° angle to the sample liquid.

3. Meter Systems

   a. The meter might measure the signal from the scattered light intensity only.

   b. The meter might measure the signal from a ratio of the scattered light versus light transmitted directly through the sample to a photocell.

4. Meter Scales and Calibration

   a. The meter may already be calibrated in NTUs. In this case, at least one standard is run in each instrument range to be used in order to check the accuracy of the calibration scales.

   b. If a pre-calibrated scale is not supplied, a calibration curve is prepared for each range of the instrument by using appropriate dilutions of the standard turbidity suspension.

C. EPA Specifications for Instrument Design(7)

Even when the same suspension is used for calibration of different nephelometers, differences in physical design of the turbidimeters will cause differences in measured values for the turbidity of the same sample. To minimize such differences, the following design variables have been specified by the U. S. Environmental Protection Agency.

1. Defined Specifications

   a. Light Source

   Tungsten lamp operated at not less than 85% of rated voltage and at not more than rated voltage.

   b. Distance Traveled by Light

   The total of the distance traversed by the incident light plus scattered light within the sample tube should not exceed 10 cm.

   c. Angle of Light Acceptance of the Detector

   Detector centered at 90° to the incident light path and not to exceed ± 30° from 90°.

   (Ninety degree scatter is specified because the amount of scatter varies with size of particles at different scatter angles).

   d. Applicable Range

   The maximum turbidity to be measured is 40 units. Several ranges will be necessary to obtain adequate coverage. Use dilution for samples if their turbidity exceeds 40 units.

2. Other EPA Design Specifications

   a. Stray Light

   Minimal stray light should reach the photocell(s) in the absence of turbidity.

Some causes of stray light reaching the photocell(s) are:

1) Scratches or imperfections in glass cell windows.
2) Dirt, film or condensation on the glass.
3) Light leakages in the instrument system.

A schematic of these causes is shown in Figure 3.

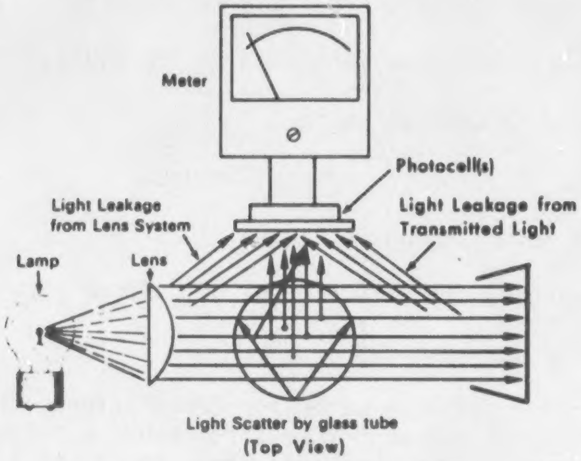

Meter
Photocell(s)
Light Leakage from Lens System
Light Leakage from Transmitted Light
Lamp
Lens
Light Scatter by glass tube
(Top View)

Figure 3 NEPHELOMETER
SOURCES OF STRAY LIGHT

Stray light error can be as much as 0.5 NTU. Remedies are close inspection of sample cells for imperfections and dirt, and good design which can minimize the effect of stray light by controlling the angle at which it reaches the sample.

b. Drift

The turbidimeter should be free from significant drift after a short warm-up period. This is imperative if the analyst is relying on a manufacturer's solid scattering standard for setting overall instrument sensitivity for all ranges.

c. Sensitivity

In waters having turbidities less than one unit, the instrument should detect turbidity differences of 0.02 unit or less. Several ranges will be necessary to obtain sufficient sensitivity for low turbidities.

3. Examples of instruments meeting the specifications listed in 1 and 2 above include:

   a. Hach Turbidimeter Model 2100 and 2100A.

   b. Hydroflow Instruments DRT 100, 200, and 1000.

4. Other turbidimeters meeting the listed specifications are also acceptable.

D. Sources of Error

1. Sample Cells
   - a. Discard scratched or etched cells.
   - b. Do not touch cells where light strikes them in instrument.
   - c. Keep cells scrupulously clean, inside and out.(8)
     - 1) Use detergent solution.
     - 2) Organic solvents may also be used.
     - 3) Use deionized water rinses.
     - 4) Rinse and dry with alcohol or acetone.

2. Standardizing Suspensions(7)
   - a. Use turbidity - free water for preparations. Filter distilled water through a 0.45μm pore size membrane filter if such filtered water shows a lower turbidity than the distilled water.
   - b. Prepare a new stock suspension of Formazin each month.
   - c. Prepare a new standard suspension and dilutions of Formazin each week.

3. Sample Interferences
   - a. Positive
     - 1) Finely divided air bubbles
   - b. Negative
     - 1) Floating debris
     - 2) Coarse sediments (settle)
     - 3) Colored dissolved substances (absorb light)

E. Reporting Results(7)

NTU	RECORD TO NEAREST
0.0-1.0	0.05
1-10	0.1
10-40	1
40-100	5
100-400	10
400-1000	50
>1000	100

F. Precision and Accuracy(7)

1. In a single laboratory (EMSL), using surface water samples at levels of 26, 41, 75 and 180 NTU, the standard diviations were ±0.60, ±0.94, ±1.2 and ±4.7 units, respectively.
2. Accuracy data is not available at this time.

V. STANDARD SUSPENSIONS AND RELATED UNITS(9)

One of the critical problems in measuring turbidity has been to find a material which can be made into a reproducible suspension with uniform sized particles. Various materials have been used.

A. Natural Materials

1. Diatomaceous earth
2. Fuller's earth
3. Kaolin
4. Naturally turbid waters.

Such suspensions are not suitable as reproducible standards because there is no way to control the size of the suspended particles.

B. Other materials

1. Ground glass
2. Microorganisms
3. Barium Sulfate
4. Lates spheres

Suspensions of these also proved inadequate.

C. Formazin

1. A polymer formed by reacting hydrazine sulfate and hexamethylenetetramine sulfate.

2. It is more reproducible than previously used standards. Accuracy of ± one percent for replicate solutions has been reported.

3. In 1958, the Association of Analytical Chemists initiated a standardized system of turbidity measurements for the brewing industry by:

   a. Defining a standard formula for making stock Formazin solutions and

   b. Designating a unit of measurement based on Formazin, i.e., the Formazin Turbidity Unit (FTU).

4. During the 1960's Formazin was increasingly used for water quality turbidity testing. It is the currently recognized standard for compliance turbidity measurements.

D. Units

1. At first results were translated into Jackson Turbidity Units (JTU). However, the JTU was derived from a visual measurement using concentrations (mg/liter) of silica suspensions prepared by Jackson. They have no direct relationship to the intensity of light scattered at 90 degrees in a nephelometer.

2. For a few years, results of nephelometric measurements using specified Formazin standards were reported directly as Turbidity Units (TUs).

3. Currently, the unit used is named according to the instrument used for measuring turbidity. Specified Formazin standards are used to calibrate the instrument and results are reported as Nephelometric Turbidity Units (NTUs).

VI. SUMMARY

The importance of residual chlorine determination can be seen in its possible effect on the health of the consumers. The Criteria and Procedures for Laboratory Certification suggests that some form of quality assurance should be instituted on a state level to assure valid data for both the chlorine and turbidity measurements. The comments on the public responses to the proposed Interim Primary Regulations also suggests some form of quality assurance on the state level to be instituted. Consequently, the Regional Certification team should point out to the principal laboratories the importance of some kind of effort being instituted. States might wish to offer some kind of formal training effort as part of the approval mechanism for the operators doing the chlorine and/or turbidity measurements.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

COLIFORM TEST BY THE MULTIPLE DILUTION TUBE METHOD (MPN)

as applied in

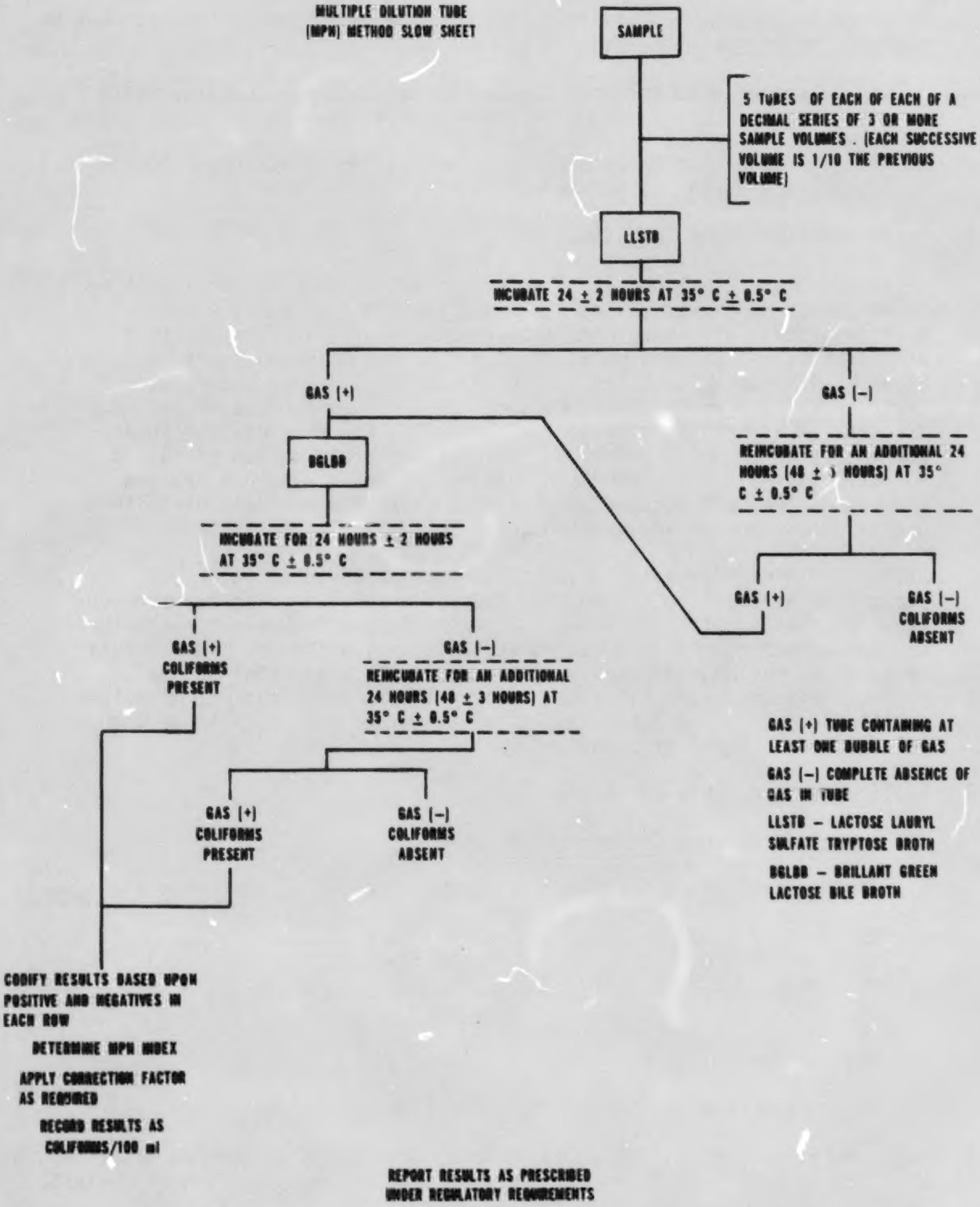
WATER TREATMENT FACILITIES
WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U. S. Environmental Protection Agency

BA.MET.lab.WMP.4.5.78

COLIFORM TEST

MULTIPLE DILUTION TUBE (MPN) METHOD SLOW SHEET


SAMPLE
5 TUBES OF EACH OF EACH OF A DECIMAL SERIES OF 3 OR MORE SAMPLE VOLUMES . (EACH SUCCESSIVE VOLUME IS 1/10 THE PREVIOUS VOLUME)
LLSTB
INCUBATE 24 ± 2 HOURS AT 35° C ± 0.5° C
GAS (+)
GAS (–)
BGLBB
REINCUBATE FOR AN ADDITIONAL 24 HOURS (48 ± 3 HOURS) AT 35° C ± 0.5° C
INCUBATE FOR 24 HOURS ± 2 HOURS AT 35° C ± 0.5° C
GAS (+)
GAS (–) COLIFORMS ABSENT
GAS (+) COLIFORMS PRESENT
GAS (–)
REINCUBATE FOR AN ADDITIONAL 24 HOURS (48 ± 3 HOURS) AT 35° C ± 0.5° C
GAS (+) COLIFORMS PRESENT
GAS (–) COLIFORMS ABSENT
CODIFY RESULTS BASED UPON POSITIVE AND NEGATIVES IN EACH ROW
DETERMINE MPN INDEX
APPLY CORRECTION FACTOR AS REQUIRED
RECORD RESULTS AS COLIFORMS/100 ml
REPORT RESULTS AS PRESCRIBED UNDER REGULATORY REQUIREMENTS

GAS (+) TUBE CONTAINING AT LEAST ONE BUBBLE OF GAS

GAS (–) COMPLETE ABSENCE OF GAS IN TUBE

LLSTB – LACTOSE LAURYL SULFATE TRYPTOSE BROTH

BGLBB – BRILLANT GREEN LACTOSE BILE BROTH

WATER MONITORING PROCEDURE: Coliform Test by the Multiple Dilution Tube (MPN) Method

1. Analysis Objectives:

In water treatment plant quality control, the objective of the test may be one or both of the following:

a. To determine whether water treatment plant influent quality meets requirements set by law or regulatory authority.

b. To determine water body quality as pertaining to upstream flow in a sanitary survey to locate source of excessive counts.

2. Brief Description of Analysis:

Three or more decimal series dilutions of a sample (For example: Five fermentation tubes with 10 ml portions, another five tubes with 1 ml portions, etc.) are inoculated into lactose lauryl sulfate tyrptose broth (LLSTB) and incubated at 35° C ± 0.5° C. After 24 hours and again at 48 hours, the LLSTB tube cultures are examined and results recorded for gas production. Cultures showing gas production are transferred at each examination interval to BGLBB fermentation tubes and incubated at 35° C ± 0.5° C. BGLBB tubes are examined at 24 and 48 hour intervals for presence of gas and those showing gas are considered gas (+) and containing coliforms while those completely without gas as gas (-) or not containing coliforms.

At the end of the overall incubation period, individual tubes are summarized as positive or negative and these results coded to represent rows of the inoculation series. A Table of Most Probable Numbers (MPN) is used with properly selected codes to determine the MPN Index. This Index is corrected, if necessary, to agree with the actual sample volumes indicated (the Table is based on 10 ml; 1 ml; and 0.1 ml volumes for the series). The final results are recorded and reported as the coliforms per 100 ml of sample.

3. Applicability of this Procedure:

a. Range of Coliform Concentration

If these dilutions are used	These ranges of Coliforms are covered
10; 1; 0.1; 0.01	> 2 to ≥ 24,000
1; 0.1; 0.01; 0.001	20 to ≥ 240,000
0.1; 0.01; 0.001; 0.0001	200 to ≥ 2,400,000
etc.	etc.

b. Pretreatment of Samples

In accordance with Standard Methods, 14th ed. (p. 904).

This procedure conforms to the Standard Total Coliform MPN Tests as described in Standard Methods for the Examination of Water and Wastewater, 14th ed., (1975), p. 916 ff.

WATER MONITORING PROCEDURE: Coliform Test by the Multiple Dilution Tube (MPN) Method

Equipment and Supply Requirements

A. Capital Equipment:

1. Autoclave, providing uniform temperatures up to and including 121° C, equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperature within 30 minutes
2. Balance, 0.1 g sensitivity at load of 150 g
3. Air Incubator to operator at 35° C ± 0.5° C
4. Oven, *hot-air sterilizing, to give uniform temperatures and with suitable thermometer to register accurately in range of 160-180° C
5. pH Meter, accurate to at least 0.1 pH unit, with standard pH reference solutions(s)
6. Water distillation apparatus, (glass or block tin), or source of distilled water suitable for bacteriological operations

B. Reusable Supplies:

1. Apron or coat suitable for laboratory
2. Baskets, wire for discarded cultures
3 Bottles, dilution*, 6-oz. screw caps, with 99 ml volume level etched on one side
4. Bottles, sample*, preferred characteristics being 250 ml (6-8 oz.), wide mouth, glass stopper
5. Burner, gas, Bunsen burner type
6. Cans, pipet, aluminum or steel; not copper (If plastic, or other type of prepackaged disposable pipets are used, this item is unnecessary.)
7. Metal caps* to fit 18 and 25 mm culture tubes
8. Pan, to receive discarded contaminated pipets and glassware (must contain disinfectant before use)
9. Inoculation loop, 3 mm diameter loop of nichrome or platinum-iridium wire, 26 B&S gauge, in holder
10. Pipets*, 1 ml, with 0.1 ml graduations, Mohr type preferred, sterile, cotton plugged, glass or disposable plastic
11. Pipets*, 10 ml, with 1.0 ml graduations, Mohr type preferred, sterile, cotton plugged, glass or disposable plastic
12. Racks, culture type*, 10 x 5 openings, to accept tubes at least 25 mm in diameter
13. Sponge, for cleaning desk top
14. Tubes, culture*, 150 x 25 mm
15. Tubes, culture*, 150 x 18 mm
16. Tubes, fermentation*, 75 x 10 mm vials to be inverted in culture tubes

C. Consumable Supplies:

1. Distilled water, suitable for bacteriological cultures (note distillation apparatus required in capital equipment)
2. BGLBB (Brilliant Green Lactose Bile Broth), dehydrated (recommend purchase of 1/4 lb. units)
3. Lactose Lauryl Sulfate Tryptose Broth, dehydrated (recommend purchase of 1 lb. units)
4. Postasium Dihydrogen Phosphate (KH_2PO_4) (recommend purchase of 1/4 lb. units)

C. Consumable Supplies (Continued):

5. Disinfectant, for bench tops. (Use household bleach solution prepared according to instructions on bottle)
6. Wax pencils (recommend soft wax equivalent to Blaisdell 169T)
7. EDTA (ethylene dinitrilotetraacetic acid)
8. Sodium thiosulfate ($Na_2S_2O_3.5\ H_2O$)

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*Items marked are needed in quantities or require size or space allowances which cannot be specified here, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of "Standard Methods for the Examination of Water and Wastewater."

WATER MONITORING PROCEDURE: Coliform Test by the Multiple Dilution Tube (MPN) Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures		Aa. All pretest procedures completed before starting other first-day procedures.	V.A.1 (p. 9-42)
1. 35° C Incubator Set-up, Adjustment	1. Place 35° C incubator in permanent location.	1a. Out of drafts or places where it will be in sunlight part of day. 1b. Location convenient to laboratory bench. 1c. Convenient source of electric power.	V.A.1.1 (p. 9-42)
	2. Install thermometer.	2a. Thermometer functions at least in 30°-40° C range and has intervals of 0.5° or less indicated. Meets NBS standards. 2b. Location should be central in incubator. 2c. Mercury bulb thermometer should be fitted with cork or rubber stopper and mounted in small bottle filled with liquid (glycerine, water, or mineral oil).	V.A.1.2 (p. 9-42)
	3. Install shallow pan of water in bottom of incubator.	3a. In most laboratory incubators a pan having about 1 square foot of area, with water about 1 inch deep, is satisfactory. 3b. Maintains condition of saturated relative humidity, required in bacteriological incubator. 3c. Requires daily check, with addition of water as necessary, to keep water in pan at all times.	V.A.1.3 (p. 9-42)
	4. Connect incubator to electric power source.	4a. Many incubators have pilot light to indicate power turned on.	
	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for method temperature adjustment. 5b. Operation must be at 35° ± 0.5° C. 5c. Allow about 1 hour between adjustments.	V.A.1.5 (p. 9-42)
	6. Operate bacteriological incubator continuously.	6a. Requires daily check with written temperature record, with adjustment and water addition as necessary.	V.A.1.6 (p. 9-42)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued) 2. Oven, Sterilizer Set-up	1. Place oven sterilizer in permanent location.	1a. Convenient to source of electric power usually on table or bench.	V.A.2.1-5 (p. 9-43)
	2. Install thermometer.	2a. Should indicate the 160° - 180° C range, be accurate within this interval, and be marked in 1.0 degree intervals.	
	3. Connect oven sterilizer to power source and turn on.	3a. Usually has pilot light to indicate power on.	
	4. Adjust temperature to stabilize at required temperature.	4a. Operated as near to 170° C as possible; not lower than 160° or higher than 180° C.	
	5. Operate oven sterilizer only when needed. Turn off when not in use.	5a. Turned ON in advance of need to permit reaching required temperature before introducing material to be sterilized. 5b. Oven sterilizer used to sterilize dry glassware, metal objects. 5c. Oven sterilizer not used with culture media, solution, plastics, rubber objects, or with anything containing or including these. 5d. Paper-wrapped glass pipets may be sterilized in oven sterilizer.	
3. Autoclave Set-up	1. Install and operate autoclave according to manufacturer's instructions.	1a. Autoclaves extremely variable in design and operation; also, potentially dangerous. 1b. Used to sterilize objects made of, or including liquids, rubber, culture media. 1c. Glassware may be autoclave sterilized but must be dried afterward. 1d. Most plastics not sterilized in autoclave; plastics usually require chemical sterilizers. 1e. Autoclave usually operated at 121° C for 15 min. 1f. Sterilized media must be removed from autoclave as soon as possible after autoclave is reopened.	V.A.3.1 (p. 9-43)

WATER MONITORING PROCEDURE: Coliform Test by the Multiple Dilution Tube (MPN) Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)			
4. Water Distillation Equipment	1. Install and operate in accordance with manufacturer's instructions.	1a. Must produce distilled water meeting quality requirements for bacteriological tests.	V.A.4.1-2 (p. 9-43)
	2. Operate continuously or intermittently as required to maintain adequate supplies of distilled water.	2a. Reserve supplies kept in borosilicate glass carboys or in plastic carboys made of material which will not dissolve substances which will affect growth of bacteria. 2b. Same distillation apparatus used for bacteriological purposes may be used for chemical reagents.	
5. pH Meter	1. Have unit available and operate in accordance with procedures described in other lab procedures.	1a. Unit for pH check on finished culture media. 1b. Used in preparation of stock solution of potassium dihydrogen phosphate.	V.A.5.1 (p. 9-44)
6. Glassware	1. Wash all glassware in hot detergent solution.	1a. Nontoxic detergent 1b. Be sure all contents and markings are washed away.	V.A.6.1-4a (p. 9-44)
	2. Rinse at least once in hot tap water.		
	3. Rinse in distilled water, at least 6 successive times and,		
	4. Dry in air.	4a. No visible spots or scum; glass should be clean, and sparkling. 4b. Glassware suitable for use in bacteriological operations.	V.A.6.1-4b (p. 9-44)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	The following special conditions may apply to the sample to be analyzed: If the sample is chlorinated influent which contains copper, zinc, or heavy metals, do operating procedures A.7, A.8 and A.9 completely. If the sample is unchlorinated influent which contains copper, zinc, or heavy metals, eliminate steps A.7 and A.9.1. If the sample is chlorinated influent which does not contain copper, zinc, or heavy metals, eliminate steps A.8 and A.9.2. If the sample is unchlorinated and contains no copper, zinc, or heavy metals, eliminate steps A.7, A.8, A.9.1 and A.9.2.		
7. Sodium Thiosulfate Solution	1. Weigh 10.0 grams of sodium thiosulfate.	1a. Used for dechlorination of samples. 1b. Use of trip balance accepted.	
	2. Dissolve in 50-60 ml distilled water.	2a. 100 ml graduated cylinder satisfactory.	
	3. Add distilled water to bring final volume to 100 ml.		
	4. Transfer to labeled bottle.	1a. Labeled as 10% sodium thiosulfate and stored in refrigerator.	
8. Ethylenedinitrilotetraacetic Acid (EDTA) Solution	1. Weigh 15.0 grams of EDTA.	1a. Used for water samples high in copper or zinc or wastewater samples high in heavy metals. 1b. Use of trip balance accepted.	
	2. Dissolve in 50-60 ml distilled water.	2a. A 100 ml graduated cylinder is satisfactory.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	3. Add distilled water to bring final volume to 100 ml.		
	4. Transfer to labeled clean bottle.	4a. The bottle should be labeled as 15% Ethylenedinitrilotetraacetic acid (EDTA) and stored in refrigerator.	
9. Sample Bottle Preparation	1. Deliver 0.1 ml or .2 ml of 10% sodium thiosulfate solution to each sample bottle. (.1 ml to 4 ounce or 120 ml size and .2 ml to 6-8 ounce or 250 ml size).	1a. Use 1 ml pipet. 1b. Provides adequate sodium thiosulfate for neutralizing chlorine in sample. 1c. Return stock sodium thiosulfate solution to refrigerator.	V.A.9.1-6 (p. 9-44)
	2. Deliver .3 ml or .6 ml of 15% EDTA solution to each sample bottle (.3 ml to 4 ounce or 120 ml size and .6 ml to 6-8 ounce or 250 ml size).	2a. Use 1 ml pipet. 2b. Provides adequate EDTA chelating agent for metals in sample. 2c. Return stock solution of EDTA to refrigerator.	
	3. Place cover on sample bottle.		
	4. Place paper or metal foil cover over bottle cap or stopper.	4a. Protects opening of sample bottle from accidental contamination.	
	5. Sterilize sample bottles in sterilizing oven.	5a. One hour at 170° C. (See A.2)	
	6. Store sample bottles in clean, dry place until used.		

WATER MONITORING PROCEDURE: Coliform Test by the Multiple Dilution Tube (MPN) Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued) 10. Pipet Preparation	1. Inspect the 10 ml and 1 ml pipets to be prepared for use; discard and destroy all having chipped or cracked tips.	1a. Cleanliness of pipet must be equivalent to glassware.	
	2. Insert plug of non-absorbent cotton into mouthpiece of each clean, dry pipet.	2a. For protection of user when pipetting sample. 2b. Cotton plug must be tight enough to prevent easy removal, either by the pipetting action or by handling, and yet loose enough to permit easy air movement through the plug.	V.A.10.1-6 (p. 9-44)
	3. Place a layer of glass wool or several layers of paper padding in bottom of pipet can.	3a. For protection of pipet delivery tips.	
	4. Place 12-24 pipets of the same size in each pipet can, delivery tip down. Mark cans as either 10 ml or 1 ml.	4a. Orientation permits removal of sterile pipets from can without contamination by user.	
	5. Sterilize cans of pipets in oven.	5a. 1 hour at 170° C. (See A.2 of procedures)	
	6. Store cans in clean, dry place until used.	6a. Laboratory cabinet or drawer recommended.	
	7. When can of pipets is opened for first use, pass the exposed ends of the pipets through flame, slowly.	7a. Burns off excess cotton sticking out of pipet mouthpiece. 7b. Cover kept on can at all times except when samples are being inoculated.	V.A.10.7 (p. 9-44)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued) 11. Dilution Water Blanks	1. Prepare stock solution of potassium dihydrogen phosphate (KH_2PO_4); dissolve 34.0 grams of the KH_2PO_4 in 500 ml distilled water. Adjust to pH 7.2 with 1N NaOH, and dilute to 1 liter with distilled water.	1a. Distilled water may be measured in 500 ml graduated cylinder. 1b. Finished solution labeled "Stock KH_2PO_4 for Dilution Water." 1c. Stored in refrigerator. 1d. Discard stock solution and prepare new solution if mold appears.	V.A.11.1.1d (p. 9-44)
	2. Prepare stock solution of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) by dissolving 50 grams of this chemical in 500-600 mls of distilled water and, after complete dissolving, bring the final volume to 1 liter in a volumetric flask.		
	3. Prepare working solution of dilution water by adding 1.25 ml KH_2PH_4 and 5 ml of the magnesium sulfate stock solution to each liter of distilled water to be made up as dilution water.	3a. 5 ml pipet satisfactory for 1 liter amounts of dilution water. 10 ml pipet better when several liters are being made. 3b. 1-liter graduated cylinder satisfactory for measurement of distilled water. 3c. Use separate pipets for each solution to prevent contamination.	
	4. Deliver enough working solution to each dilution water bottle so that after sterilization the bottles will contain 99 ± 2 ml of dilution water.	4a. 100 ml graduated cylinder ordinarily satisfactory. Pipetting machine desirable but not mandatory. 4b. Amount cannot be stated exactly, as sterilization evaporation differs from one autoclave to another. Commonly, about 102 mls are required.	V.A.11.4 (p. 9-45)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	5. Place caps on dilution bottles loosely.		V.A.11.5 (p. 9-45)
	6. Sterilize in autoclave.	6a. 15 minutes at 121° C. Use "slow-vent" mode of steam evacuation.	V.A.11.6 (p. 9-45)
	7. Promptly remove from autoclave, tighten bottle caps, cool to room temperature.		
	8. Store in cool place.	8a. Dilution water ready for use. May be stored indefinitely in screw-capped bottles.	V.A.11.8 (p. 9-45)
12. Preparation of Lactose Lauryl Sulfate Tryptose Fermentation Broth (LLSTB)	Single-Strength Medium 1. Weigh 35.6 grams of dehydrated Lactose Lauryl Sulfate Tryptose Broth. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media takes moisture out of air; can become caked. 1b. Caked media unsatisfactory; should be discarded.	
	2. Dissolve in 1 liter distilled water.	2a. Gentle heat (no boiling) if necessary to complete dissolving medium. Usually a vigorous agitation will completely dissolve the medium.	
	3. Place 10.5 ml of the solution of prepared LLSTB in each culture tube.	3a. Use 150 x 18 mm tubes. 3b. A 25 ml pipet, automatic pipetter, or funnel hose and pinchcock assembly are acceptable. 3c. Accuracy of delivery: ± 0.5 ml. 3d. Approximately 90 tubes will be necessary. This will suffice for 6 tests based upon procedures of this WMP (Water Monitoring Procedure).	V.A.12.3b (p. 9-45)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	4. Insert one fermentation vial into each tube of medium, open end down.	4a. Tubes and vials previously washed as indicated (A.6.1-4.) 4b. Use 75 x 10 mm tubes.	
	5. Place tube cap on each tube of culture medium.	5a. After all tubes have been filled and have individual vial.	
	6. Sterilize in autoclave.	6a. Within 1 hour after medium is prepared. 6b. Sterilization at 121° C for 15 minutes. 6c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" mode of steam removal.	
	7. Cool medium to room temperature.	7a. Medium ready for use when cool and individual vials are completely filled with fluid. No bubbles must be present.	
	8. Check pH of finished medium.	8a. Should be pH 6.7 - 6.9.	
	9. If final pH is not satisfactory, discard medium and prepare new batch with pH adjustment before sterilization.	9a. pH value ordinarily drops about 0.2 pH unit.	
	10. Store medium in cool dark place.	10a. Not in refrigerator. Usually in laboratory cabinet in darkness. 10b. May be stored up to 1 week if evaporation is not more than 10% in loose-fitting capped tubes. With screw-capped tubes should be held no longer than 3 months.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	Increased Strength Medium 11. Weigh 53.4 grams of dehydrated Lactose Lauryl Sulfate Tryptose Broth. Close cover of bottle of dehydrated medium tightly after removal.	11a. Dehydrated media takes moisture out of the air; can become caked. 11b. Caked media unsatisfactory; should be discarded.	
	12. Dissolve in 1 liter distilled water.	12a. Gentle heat (no boiling) if necessary to complete dissolving medium. Usually a vigorous agitation will completely dissolve the medium.	
	13. Place 20.5 ml of the solution of prepared LLSTB in each culture tube.	13a. Use 150 x 25 mm tubes. 13b. 25 ml pipets, automatic pipetter, or funnel hose and pinchcock assembly are acceptable. 13c. Accuracy of delivery: ± 0.5 ml. 13d. Approximately 45 tubes will be necessary. This will suffice for 9 tests based upon procedures of this WMP.	V.A.12.3b (p. 9-45)
	14. Continue step sequence as in 12.4-10 to complete preparation of increased strength LLSTB.		
13. Preparation of Brilliant Green Lactose Bile Broth (BGLBB)	1. Weigh 40.0 grams of dehydrated Brilliant Green Lactose Bile Broth. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media takes moisture out of the air: can become caked. 1b. Caked media unsatisfactory; should be discarded.	
	2. Dissolve in 1 liter distilled water.	2a. Gentle heat (no boiling) if necessary to complete dissolving medium. Usually a vigorous agitation will completely dissolve the media.	

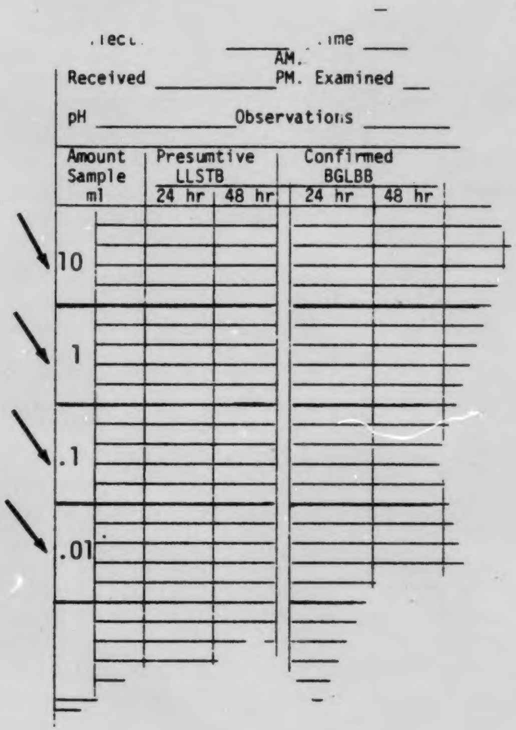
WATER MONITORING PROCEDURE: Coliform Test by the Multiple Dilution Tube (MPN) Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	3. Place 10.5 ml of the solution of prepared BGLBB in each culture tube.	3a. Use 150 x 18 mm tubes 3b. A 25 ml pipet, automatic pipetter or funnel hose and pinchcock assembly are acceptable. 3c. Accuracy of delivery ± 0.5 ml. 3d. Approximately 90 tubes will be necessary.	V.A.12.3b (p. 9-45)
	4. Insert one fermentation vial into each tube of medium, open end down.	4a. Tubes and vials previously washed as indicated (A.6.1-4). 4b. Use 75 x 10 mm tubes.	
	5. Place cap on each tube of culture medium.	5a. After all tubes have been filled and have individual vial.	
	6. Sterilize in autoclave.	6a. Within 1 hour after medium prepared. 6b. Sterilization at 121° C for 15 minutes. 6c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" mode of steam removal.	
	7. Cool medium to room temperature.	7a. Medium ready for use when cool and individual vials are completely filled with fluid. No bubbles must be present.	
	8. Check pH of finished medium.	8a. Should be 7.1 - 7.3.	
	9. If final pH not satisfactory, discard medium and prepare new batch with pH adjustment before sterilization.	9a. pH value ordinarily drops about 0.2 pH unit.	

WATER MONITORING PROCEDURE: Coliform Test by the Multiple Dilution Tube (MPN) Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	10. Store medium in cool dark place.	10a. NOT in refrigerator. Usually in laboratory cabinet in darkness. 10b. May be stored up to 1 week if evaporation not more than 10% in loose-fitting capped tubes. With screw-capped tubes should be held no longer than 3 months.	
14. Final Equipment and Supply Check	1. Check to be sure that all equipment and supplies, solutions, and prepared media are ready before starting sample examination.	1a. Check general list of equipment and supplies. 1b. Each test requires (with 4 sample volumes per test): 5 tubes 1.5X LLSTB (150 x 25 mm tubes) 15 tubes 1X LLSTB (150 x 18mm tubes) 10-15 tubes BGLBB 1 sample bottle, sterile 1 10 ml pipet, sterile 2 1 ml pipets 1 99 ml sterile dilution blank.	
	2. Make preparations or adjustments as necessary before starting test.		
B. First-day Procedures 1. Equipment Maintenance	1. Check, record, and adjust incubator temperature.	1a. See A.1.1-6	
	2. Add water to pan in incubator as necessary.		
2. Sample Collection	1. Collect sample.		
	2. Record sampling information.	2a. Most plants have sample tag of some type which includes such information as date, time, place of sampling, name of sample collector, and other information as may be required.	
	3. Transport sample to laboratory.	3a. Taken to laboratory without delay. 3b. Samples iced if delay of starting sample test is greater than one hour. No more than 6 hours of transportation time is allowed.	

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>B. First-day Procedures (Continued)
3. Preparation of Laboratory Data Sheet.</td><td>1. Fill in data sheet to show sample information.</td><td>1a. Needed information should be on sample collection tag.
1b. Most data sheets show at least source, date, time of collection, name of sampler, name of analyst, laboratory sample number assigned.</td><td>VII.B.3.1
(p. 9-46)</td></tr>
<tr><td></td><td>2. Select sample inoculation volumes.</td><td>2a. According to coliform density range predicted for the sample.
2b. For coliforms per 100 ml in the range
<table>
<tr><th>from</th><th>to</th><th colspan="4">inoculate 5 tubes each of ml</th></tr>
<tr><td>2 -</td><td>16,000</td><td>10.0,</td><td>1.0,</td><td>0.1,</td><td>0.01</td></tr>
<tr><td>20 -</td><td>160,000</td><td>1.0,</td><td>0.1,</td><td>0.01,</td><td>0.001</td></tr>
<tr><td>200 -</td><td>1,600,000</td><td>0.1,</td><td>0.01,</td><td>0.001,</td><td>0.0001</td></tr>
<tr><td>2,000 -</td><td>16,000,000</td><td>.01,</td><td>.001,</td><td>.0001,</td><td>.00001</td></tr>
<tr><td>20,000 -</td><td>160,000,000</td><td>.001,</td><td>.0001,</td><td>.00001,</td><td>.000001</td></tr>
</table>
2c. For chlorinated influents, 1.0, 0.1, 0.01, and 0.001 ml sample portions are recommended.
2d. For raw (untreated) sewage, use sample portions of 0.0001, 0.00001, 0.000001, and 0.0000001 ml.
2e. For other waters, other combinations of sample volumes may be required, particularly in environmental waters receiving raw or incompletely treated sewage. It may be necessary to conduct exploratory tests.
2f. For purposes of this WMP, the selected volumes will be:
10.0; 1.0; 0.1; and 0.01</td><td>VII.B.3.2
(p. 9-46)</td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-day Procedures (Continued)	3. Enter information in laboratory data sheet to show sample inoculation volume for each series (row) of 5 tubes.	3a. Recommend showing sample inoculation volumes in ml or decimal amounts. 	
4. Lab Bench Disinfection	1. Disinfect laboratory bench; wipe dry.	1a. Sponge and disinfenctant; paper toweling.	

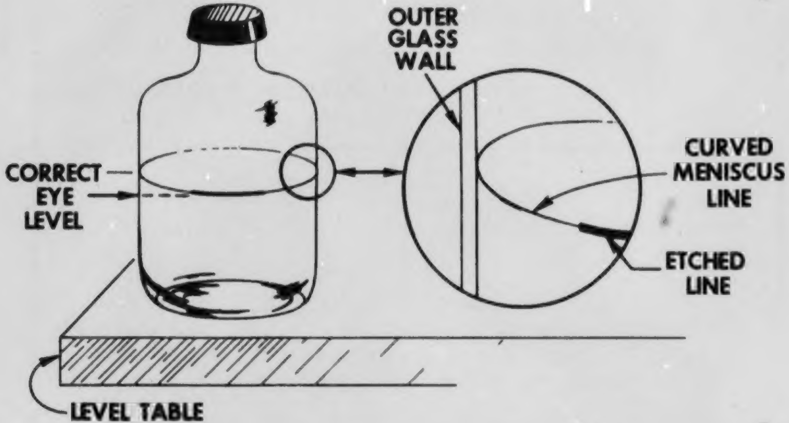
.lect. ______ AM. .ime ____

Received ___________ PM. Examined __

pH _____________ Observations ________

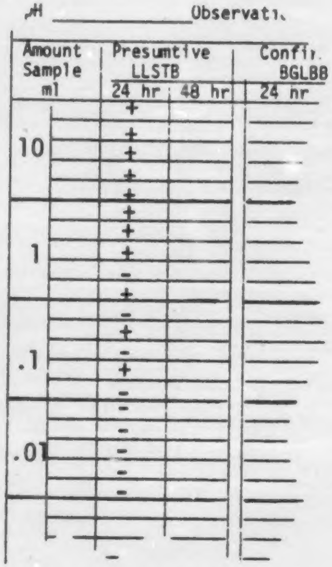
Amount Sample ml	Presumtive LLSTB 24 hr	Presumtive LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr
10				
1				
.1				
.01				

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-day Procedures (Continued)			
5. Assembly and Labeling of Culture Medium	1. Place 5 tubes of Lactose Lauryl Sulfate Tryptose Broth (LLSTB) in each of 4 rows in culture tube rack. (20 total tubes)	1a. First row of 5 tubes to contain 1.5X LLSTB (increased strength broth) and the next 3 rows to contain the single strength medium.	
	2. Label tubes of culture medium to show sample number, sample volume, and position of tube in the series of 5 tubes per sample volume.	2a. Use labeling code which allows instructor to follow manipulation of tubes by trainee throughout procedure. 2b. Label every tube. Only the experienced worker should take short-cuts in labeling. 2c. Use wax pencil. Soft wax equivalent to Blaisdell 169T is suggested.	VII.B.5.2 (p. 9-47)
6. Sample Inoculations	Row 1 1. Shake sample vigorously.	1a. At least 25 shakes over space of at least 1 foot in 10 seconds or less.	VII.B.6 I.B.6.1.1 (p. 9-48) (p. 9-36)
	2. Deliver 10 ml of sample into each of tubes in Row 1.	2a. Use the same originally sterile 10 ml pipet for each of the 5 tubes. 2b. Discard pipet into discard tray.	
	Row 2 3. Deliver 1 ml of sample into each of tubes of Row 2.	3a. Use the same originally sterile 1 ml pipet for each of the 5 tubes. 3b. Do not contaminate (bench-top, hands, etc.) pipet as it will be needed further.	
	Row 3 4. Deliver 0.1 ml of sample into each of tubes of Row 3.	4a. Use the 1 ml pipet (as used for Row 2) to deliver 0.1 ml into each of the 5 tubes. 4b. Do not contaminate (bench-top, hands, etc.) pipet as it will be needed further.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-day Procedures (Continued)	Row 4 5. Deliver 1.0 ml of sample into 99 ml dilution blank.	5a. Water within 99 ml blank must have meniscus in line with etched bottle marking before sample delivery.  5b. Discard 1 ml pipet into discard tray.	
	6. Shake dilution blank vigorously.	6a. As previously described.	
	7. Deliver 1 ml of dilution blank water into each of tubes of Row 4.	7a. Use a sterile 1 ml pipet. 7b. Discard pipet into discard tray.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-day Procedures (Continued)			
7. Incubation	1. After completion of sample inoculation into LLSTB, shake rack of cultures gently.	1a. Mixes sample with culture medium. 1b. Avoid shaking air into fermentation vials.	
	2. Place rack(s) of cultures in incubator.	2a. 24 hours ± 2 hours at 35 ± 0.5° C.	
8. Processing Used Glassware	1. Drain sample bottles, dilution bottles, and pipets into sink.	1a. Sterilization unnecessary.	
	2. Wash and dry bottles, pipets.	2a. Meets original cleanliness requirements of glassware. 2b. Glassware ready for reuse.	
9. Lab Bench Disinfection	1. Disinfect laboratory bench top; wipe dry.	1a. Sponge, disinfectant, paper toweling.	
C. 24-Hour Procedures			
1. Equipment Maintenance	1. Check, record, and adjust incubator temperature.	1a. See A.1.1-6.	
	2. Add water to pan in incubator as necessary.		
2. Disinfection	1. Disinfect laboratory bench top; wipe dry.	1a. See B.4.1.	
3. Reading and Recording of Results	1. Remove rack(s) of cultures from incubator to lab bench.		

WATER MONITORING PROCEDURE: Coliform Test by the Multiple Dilution Tube (MPN) Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. 24-Hour Procedures (Continued)	2. Shake culture rack gently.	2a. Hastens release of gas in supersaturated cultures 2b. Must not shake air into fermentation vials.	
	3. Examine each tube for gas production and record results on data sheet.	3a. If present, gas will be trapped in the fermentation vial. 3b. Gas in any quantity is a positive test. 3c. Vials with no gas are a negative test. 3d. Each result appears on line corresponding with the tube label. 3e. All results appear under the "24" of the LLSTB column. 3f. Plus sign (+) means a gas-positive tube. 3g. Minus sign (-) means a gas-negative tube. 3h. Assume, for instruction purposes, that the following recordings result: 	III.C.3.3 (p. 9-41)

pH ______________Observati.

Amount Sample ml	Presumtive LLSTB 24 hr	48 hr	Confir. BGLBB 24 hr
10	+		
	+		
	+		
	+		
	+		
1	+		
	+		
	+		
	-		
	+		
.1	-		
	+		
	-		
	+		
	-		
.01	-		
	-		
	-		
	-		
	-		
	-		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. 24-Hour Procedures (Continued) 4. Transfers	1. Label and assemble tubes of BGLBB.	1a. One tube of each LLSTB gas-positive tube. 1b. Each BGLBB tube label corresponds with label on gas-positive LLSTB tube. 1c. Labeled BGLBB tubes assembled in a culture tube rack in same relative position as gas-positive LLSTB tubes in their rack. In our example there will be eleven tubes of BGLBB required.	
	2. Transfer each gas-positive tube of LLSTB to a labeled tube of BGLBB.	2a. Label on inoculated tube of BGLBB is the same as the label on the tube of LLSTB from which the transfer is made. 2b. 3 mm inoculation loop. 2c. Loop flame-sterilized before use and between successive transfers. 2d. One loopful per transfer. 2e. Place inoculated BGLBB tube into hole of rack previously occupied by the LLSTB tube from which the transfer was made. 2f. Place positive LLSTB tube into discard area after transfer is made. All discard tubes are to be sterilized prior to cleaning and reuse of caps and tubes.	VII.C.4.2 (p. 9-50)
	3. Return rack of tubes containing the negative LLSTB tubes and the freshly inoculated BGLBB tubes to the 35° C incubator.	3a. An additional 24 ± 2 hours at 35° ± 0.5° C.	
5. Processing Discarded Cultures	1. Sterilize discarded LLSTB tubes.	1a. Autoclave: 15 minutes at 121° C.	
	2. Remove all labels from culture tubes.	2a. Best done while still warm after autoclave.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. 24-Hour Procedures (Continued)	3. Empty sterilized cultures into sink.		
	4. Wash and dry culture tubes, fermentation vials, and tube caps.	4a. Meets original cleanliness requirements of glassware. 4b. Tubes and caps ready for reuse.	
6. Disinfection	1. Disinfect laboratory bench top; wipe dry.	1a. Sponge and disinfectant; paper toweling.	
D. 48-Hour Procedures 1. Equipment Maintenance	1. Check, record, and adjust incubator temperatures.		
	2. Add water to pan in incubator as necessary.		
2. Disinfection	1. Disinfect lab bench top; wipe dry.		
3. Reading and Recording of Results	1. Remove the rack of cultures from the incubator to lab bench.		
	2. Shake culture rack gently.		
	3. Examine each tube for gas production and record results on data sheet.	3a. LLSTB tubes will be recorded under the "48" on the LLSTB column and the BGLBB tubes under the "24" column. 3b. Any amount of gas is always considered to be a "positive" result.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. 48-Hour Procedures (Continued)		3c. Assume that our "test" now shows the following recordings:	

…servations

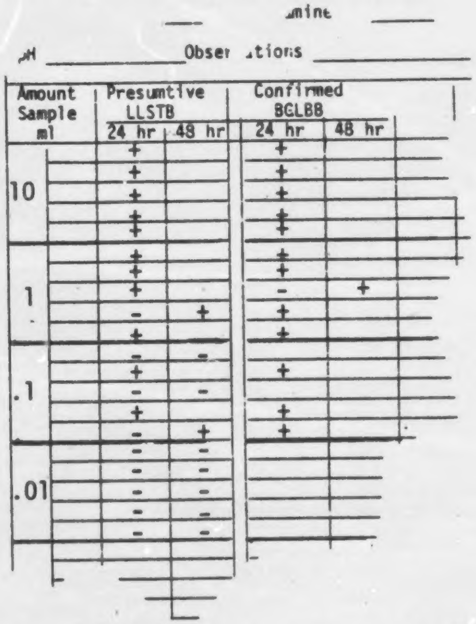
Amount Sample ml	Presumtive LLSTB 24 hr	Presumtive LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48
10	+		+	
	+		+	
	+		+	
	+		+	
	+		+	
1	+		+	
	+		+	
	+		-	
	-	+		
	+		+	
.1	-	-		
	+		+	
	-	-		
	+		+	
	-	+		
.01	-	-		
	-	-		
	-	-		
	-	-		
	-	-		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Transfers	1. Discard all of the BGLBB tubes which have the positive recordings.	1a. This will be a total of ten tubes (See data sheet recordings in D.3.3.c).	
	2. Discard all LLSTB tubes which have the negative recordings.	2a. This will be a total of seven tubes (D.3.3.c). 2b. LLSTB tubes which show no gas production within 48 hours are to be considered as not having contained coliform bacteria.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. 48-Hour Procedures (Continued)	3. Re-incubate any BGLBB tubes which were negative and assemble for transfer any positive LLSTB tubes.	3a. There will be one tube of BGLBB which must be re-incubated for an additional 24 hours at 35° ± 0.5° C. 3b. There will be two positive LLSTB tubes.	
	4. Label required tubes of sterile BGLBB tubes.	4a. Two tubes of BGLBB should be labeled to correspond to the two markings of the positive LLSTB tubes.	
	5. Transfer each of the two gas-positive LLSTB tubes to its corresponding tube of BGLBB.	5a. Use 3 mm loop which is flamed prior to entry into the LLSTB to avoid contamination or cross-contamination. 5b. Use one loopful of transfer from the LLSTB to BGLBB.	
	6. After each transfer, place LLSTB tubes in discard basket.	6a. Contaminated tubes are to be sterilized prior to cleaning operation.	
	7. Place inoculated BGLBB tubes in the 35° ± 0.5° C incubator.		
	7a.(Alternate) If no cultures for this test procedure remain to be incubated, proceed to Interpretation of Test Results and continue as directed.		
5. Processing Discarded Tubes of Media	1. Sterilize discarded media.		
	2. Remove all labels from culture tubes.		
	3. Empty sterilized cultures into sink.		

WATER MONITORING PROCEDURE: Coliform Test by the Multiple Dilution Tube (MPN) Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. 48-Hour Procedures (Continued)	4. Wash and dry culture tubes, fermentation vials, and tube caps.		
6. Disinfection	1. Disinfect laboratory bench top; wipe dry.		
E. 72-Hour Procedures 1. Equipment Maintenance	1. Check, record, and adjust incubator temperatures.		
	2. Add water to pan in incubator as necessary.		
2. Disinfection	1. Disinfect lab bench top; wipe dry.		
3. Reading and Recording of Results	1. Remove cultures from incubator to lab bench.		
	2. Shake cultures gently.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. 72-Hour Procedures (Continued)	3. Examine each tube for gas production and record results on data sheet.	3a. In our continuing example, 3 tubes of BGLBB are to be examined - one of which will be a "48" entry and the other two of the "24" column entry. Assume the following recordings: 	
	4. Incubate any cultures which are still negative if they have not been incubated a full 48 hours.	4a. Since both "24 hour" recordings of the BGLBB have become positive, NO further culturings are necessary and one could proceed with the Interpretation of Test Results instead of the 96 hour procedure.	

ɹmine

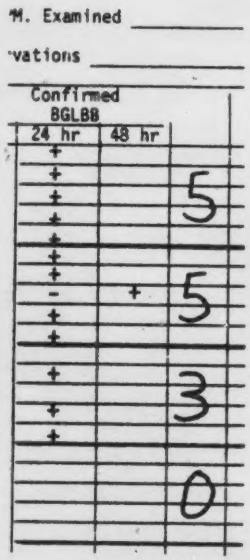
ɹH Obser ɹtions

Amount Sample ml	Presumtive LLSTB 24 hr	Presumtive LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr
10	+		+	
	+		+	
	+		+	
	+		+	
	+		+	
1	+		+	
	+		+	
	+		-	+
	-	+	+	
	+		+	
.1	-	-		
	+		+	
	-	-		
	+		+	
	-	+	+	
.01	-	-		
	-	-		
	-	-		
	-	-		
	-	-		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Processing Discarded Tubes of Media	1. Sterilize discarded tubes of media. 2. Remove all labels from tubes. 3. Empty sterilized tubes into sink.		
5. Disinfection	1. Disinfect lab bench top; wipe dry.		
F. Interpretation of Test Results	1. Determine number of BGLBB tubes which are positive for each group of five tubes of equal sample volumes.	1a. NO consideration of Presumptive Test (LLSTB) for interpretation of test results. 1b. Our example (E.3.3) shows 5 positive 1st row 5 positive 2nd row 3 positive 3rd row 0 positive 4th row	II.F.1-2 (p. 9-37)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Interpretation of Test Results (Continued)	2. Write the numbers in the data sheet.	2a. (see data sheet below)	
	3. Select the 3-digit code which applies to the number of gas-positive tubes of BGLBB.	3a. In a test involving 4 sample volumes this will be based on rows 1, 3, 3, or on rows 2, 3, 4; and 3b. If all tubes are positive in rows 1 and 2, then the 3-digit code is based on rows 2, 3, 4. 3c. In all other cases the 3-digit code is based on rows 1, 2, 3.	II.F.3 (p. 9-37)

2a.



M. Examined ______

vations ______

Confirmed BGLBB 24 hr	48 hr	
+		5
+		
+		
+		
+		
+		5
+		
-	+	
+		
+		
		3
+		
+		
+		
		0

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>F. Interpretation of Test Results (Continued)</td><td>4. Look up and record on the data sheet the MPN Index.</td><td>4a. For the given example the location of the MPN index is shown by the arrow based on the 5-3-0 code.
Table of Most Probable Numbers (MPN)
<table>
<tr><th colspan="3">No. of Tubes Giving Positive Reaction out of</th><th rowspan="2">MPN Index per 100 ml</th></tr>
<tr><th>5 of 10 ml Each</th><th>5 of 1 ml Each</th><th>5 of 0.1 ml Each</th></tr>
<tr><td>5</td><td>1</td><td>0</td><td>33</td></tr>
<tr><td>5</td><td>1</td><td>1</td><td>46</td></tr>
<tr><td>5</td><td>1</td><td>2</td><td>63</td></tr>
<tr><td>5</td><td>2</td><td>0</td><td>49</td></tr>
<tr><td>5</td><td>2</td><td>1</td><td>70</td></tr>
<tr><td>5</td><td>2</td><td>2</td><td>94</td></tr>
<tr><td>5</td><td>3</td><td>0</td><td>79</td></tr>
</table></td><td>II.F.4
(p. 9-38)</td></tr>
<tr><td></td><td>5. Divide the MPN Index by the number of mls of sample represented by the middle digit of the MPN Code. The number obtained is the MPN (Most Probable Number) per 100 ml of original sample.</td><td>5a. Calculates to be 790.</td><td>II.F.5
(p. 9-38)</td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Interpretation of Test Results (Continued)	6. Record the calculated Total Coliforms per 100 ml on the laboratory data sheet.	RESULTS: Coliform MPN 790	II.F.6 (p. 9-38)
G. Reporting of Results	1. Report results as prescribed under regulatory requirements.		

WATER MONITORING PROCEDURE: Coliform Test by the Multiple
Dilution Tube (MPN) Method

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II*	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
VII*	Field & Laboratory Analyses
VIII	Safety
IX	Records and Reports

Training guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B. 6.1.1	These MPN methods for determining bacterial numbers are based on the assumption that the bacteria can be separated from one another (by shaking or other means) resulting in a suspension of individual bacterial cells, uniformly distributed through the original sample when the primary inoculation is made. Test procedures are based on certain fundamental assumptions: a. First, even if only one living cell of the test organisms is present in the sample, it will be able to grow when introduced into the primary inoculation medium; b. Second, growth of the test organism in the culture medium will produce a result which indicates presence of the test organism; and; c. Third, unwanted organisms will not grow, or if they do grow, they will not limit growth of the test organism; nor will they produce growth effects that will be confused with those of the bacterial group for which the test is designed.	

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

F.1-2 For purely qualitative aspects of testing for indicator organisms, it is convenient to consider the tests applied to one sample portion, inoculated into a tube of culture medium, and the follow-up examinations and tests on results of the original inoculation. Results of testing procedures are definite: positive (presence of the organism-group is demonstrated or negative (presence of the organism-group is not demonstrated).

The combination of positive and negative results is used in an application of probability mathematics to secure a single MPN value for the sample.

To obtain MPN values, the following conditions must be met:

a. The testing procedure must result in one or more tubes in which the test organism is demonstrated to be present; and

b. The testing procedure must result in one or more tubes in which the test organism is not demonstrated to be present.

The MPN value for a given sample is obtained through the use of MPN Tables. It is emphasized that the precision of an individual MPN value is not great when compared with most physical or chemical determinations.

Standard practice in water tests made by most organizations is to plant five tubes in each of a series of sample increments, in sample volumes decreasing at decimal intervals.

As an example, assume that all tubes were positive for a sample portion of 10 ml, all five tubes were positive on the portions of 1 ml, three of the five 0.1 ml portions were positive, and none of the five 0.01 ml portions were positive.

The numbers, on the above example, would be 5-5-3-0.

F.3 1. Pursuing the above example, the code would be 5-3-0.

2. Selection of codes is sometimes complicated. For further information study training guide notes and cited references. Std. Meth. 14:923 ff

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
F.4	1. Appears on MPN Table (attached to this Section) 2. Pursuing the given example, the MPN Index for MPN Code 5-3-0 would be 79.	
F.5	1. As indicated above, the middle digit is 3; and it represents a sample portion of 0.1 ml. An MPN Index of 79 divided by 0.1 is 790.	
F.6	The Coliforms per 100 ml would be recorded as 790.	

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Table of Most Probable Numbers (MPN)

No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml
5 of 10 ml Each	5 of 1 ml Each	5 of 0.1 ml Each	
0	0	0	<2
0	0	1	2
0	1	0	2
0	2	0	4
1	0	0	2
1	0	1	4
1	1	0	4
1	1	1	6
1	2	0	6
2	0	0	5
2	0	1	7
2	1	0	7
2	1	1	9
2	2	0	9
2	3	0	12
3	0	0	8
3	0	1	11
3	1	0	11
3	1	1	14
3	2	0	14
3	2	1	17
3	3	0	17
4	0	0	13
4	0	1	17
4	1	0	17
4	1	1	21
4	1	2	26
4	2	0	22
4	2	1	26
4	3	0	27
4	3	1	33
4	4	0	34
5	0	0	23
5	0	1	31
5	0	2	43

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

Table of Most Probable Numbers (MPN)

No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml
5 of 10 ml Each	5 of 1 ml Each	5 of 0.1 ml Each	
5	1	0	33
5	1	1	46
5	1	2	63
5	2	0	49
5	2	1	70
5	2	2	94
5	3	0	79
5	3	1	110
5	3	2	140
5	3	3	180
5	4	0	130
5	4	1	170
5	4	2	220
5	4	3	280
5	4	4	350
5	5	0	240
5	5	1	350
5	5	2	540
5	5	3	920
5	5	4	1600
5	5	5	≥2400

C.3.3

Interpretation of results on LLSTB:

Development of gas in this medium indicates that the lactose has been fermented. Fermentation of lactose with gas production is a basic characteristic of coliform bacteria. To meet the definition of coliforms, gas must be produced from lactose within 48 hours after being placed in the incubator. If a culture develops gas only after more than 48 hours incubation, then, by definition, it is not a coliform.

Meeting previously discussed assumptions (See I.B.6.1.1) usually makes it necessary to conduct the tests in a series of stages.

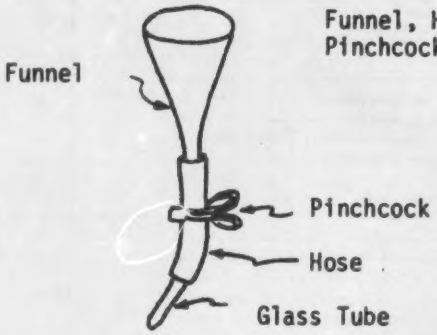
Features of a full, multi-stage test:

a. First stage: The culture medium usually serves primarily as an enrichment medium for the group tested. A good first-stage growth medium should support growth of all the living cells of the group tested, and it should include provision for indicating the presence of the test organism being studied. A first-stage medium may include some component which inhibits growth of extraneous bacteria, but this feature never should be included if it also inhibits growth of any cells of the group for which the test is designed. The Presumptive Test for the coliform group is a good example. The medium supports growth, presumably, of all living cells of the coliform group; the culture container has a fermentation vial for demonstration of gas production resulting from lactose fermentation by coliform bacteria, if present; and sodium lauryl sulfate may be included in one of the approved media for suppression of growth of certain non-coliform bacteria. This additive apparently has no adverse affect on growth of members of the coliform group in the concentrations used. If the result of the first-stage test is negative, the study of the culture is terminated, and the result is recorded as a negative test. No further study is made of negative tests. If the result of the first-stage test is positive, the culture may be subjected to further study to verify the findings of the first stage.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1	Incubator must be of sufficient size for daily work load without causing crowding of tubes to be incubated. Considerations for choice of incubator type must relate to reliability of operation and not to cost or attractiveness of equipment.	
A.1.1	Incubator should be kept out of drafts or direct sunlight in order to prevent temperature inside the incubator from changing outside the temperature range specified (35° ± 0.5°). Power supply should be selected so that there will not be too many pieces of equipment on the same circuit. Otherwise, circuits will be blown repeatedly.	Standard Methods for the Examination of Water and Wastewater, 14th ed. (1975 APHA, WPCF, AWWA, p. 880 (Hereafter referred to as: Std. Meth. 14: (page no.)
A.1.2	Mercury bulb thermometer usually used in most incubators. Recording thermometer is acceptable, but, it should be calibrated against a mercury bulb thermometer which has been certified by National Bureau of Standards. The NBS certified thermometer always should be used with its certificate and correction chart.	
A.1.3	Saturated relative humidity is required in order to make the incubation more efficient (heat is transferred to cultures faster than in a dry incubator). Furthermore, culture medium may evaporate too fast in a dry incubator.	
A.1.5	Allow enough time after each readjustment to permit the incubator to stabilize before making a new adjustment. At least one hour is suggested.	
A.1.6	Incubator temperature can be held to much closer adjustment if operated continuously. Temperature records should be kept in some form of permanent record. A temperature record book is suggested with daily recording of values. If a recording thermometer is used, the charts may be kept as permanent record; if so, be sure that the charts are properly labeled to identify the incubator and the period covered. Uniform temperature (35° C ± 0.5) is to be maintained on shelves in use.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.2.1-5	Since electric sterilizer will be operated intermittently, care should be taken that it is on a circuit which will not be overloaded when it is turned on. A time and temperature record is maintained for each sterilization cycle. Temperature recordings can be retained for records.	Std. Meth. 14:881
A.3.1	Autocalves differ greatly in design and in method of operation. Some are almost like home-style pressure cookers; others are almost fully automatic. This is a subject which requires separate instruction; and should be related to the exact make and model of equipment you will use in your own laboratory. Vertical autoclaves and household pressure cookers may be used in emergency service if equipped with pressure gages and thermometers with bulbs positioned 1 inch above the water level. However, they are not to be considered the equivalent of the general purpose steam sterilizer recommended for permanent laboratory facilities. Their small size is inadequate for large-volume work loads, and they can be difficult to regulate. The following requirements must be met regarding autoclaves or sterilizing units: a. Reaches sterilization temperature (121°C), maintains 121°C during sterilization cycle, and requires no more than 45 min. for a complete cycle. b. Pressure and temperature gages on exhaust side and an operating safety valve. c. No air bubbles produced in fermentation vials during depressurization. d. Record maintained on time and temperature for each sterilization cycle.	Std. Meth. 14:881
A.4.1-2	Distilled water in a bacteriological laboratory must not contain substances which will prevent any bacteria from growing in culture medium in which the distilled water is used or will be highly nutritive. There are procedures for testing quality of distilled water; but these should be undertaken only by professional bacteriologists or in laboratories where this is done regularly. Use only glass stills or block tin lined stills.	St. Meth. 14:645-49 14:888-891

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.5.1	pH Meter: See cited reference.	Std. Meth. 14:882
A.6.1-4a	Glassware: See cited reference on pipets and graduated cylinders, media utensils, bottles.	Std. Meth. 14:882-885
A.6.1-4b	Glassware can be checked for bacteriostatic or inhibitory residues by a bacteriological test procedure which, like the distilled water suitability test, should be undertaken only by professional bacteriologists or in laboratories where this test is done on a regular basis.	
A.9.1-6	Sample bottles: Wide-mouthed, glass-stoppered bottles suggested, but other styles acceptable. If glass-stoppered bottles are used, a strip of paper should be placed in the neck of the bottle before placing the stopper in place in preparation for sterilization. This prevents the glass stopper from "freezing" in place during sterilization. The paper strip is discarded at the time of sample collection.	Std. Meth. 14:884 14:904
A.10.1-6	Pipets: This procedure is described in terms of reusable glass pipets. However, single-service prepackaged glass or plastic pipets may be purchased and used, if preferred. In case of use of single-service pipets, they will be sterile when purchased, are used one time, and discarded immediately after use. Accordingly, in the step-by-step procedures disregard any instructions about preparation of pipets for reuse in case of using single-service pipets.	Std. Meth. 14:882-883
A.10.7	Passing the opened can of pipets through a flame burns off excess cotton wisps sticking out of the mouthpiece of the pipet. If this is not done, it is almost impossible to control sample measurement accurately. Some workers may elect to accomplish this step prior to the sterilization procedure.	
A.11.1.1d	See cited reference. In time, this solution will become mold-infested. At this time it should be discarded and a new stock solution prepared.	Std. Meth. 14:892

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.11.4	Dilution water preparation: Measurement of dilution water into bottle with a 100 ml graduated cylinder is time-consuming, but effective. An automatic pipetting machine can be considered a luxury, but is a real time-saver.	
A.11.5	If caps are not placed on bottles of dilution water loosely, they may crack in autoclave; furthermore, steam will not be able to get in contact with the material being sterilized. After sterilization, tightening caps on bottles of distilled water will permit them to be kept for long periods.	
A.11.6	Always pack material loosely and away from walls in autocalve when preparing to sterilize. Steam must flow freely around materials being sterilized.	
A.11.8	If water should evaporate noticeably or become contaminated by microbial growth, the bottle of distilled water should be discarded.	
A.12.3b	Funnel, Hose, and Pinchcock Assembly  NOTE: Unit need not be sterile for medium delivery only.	

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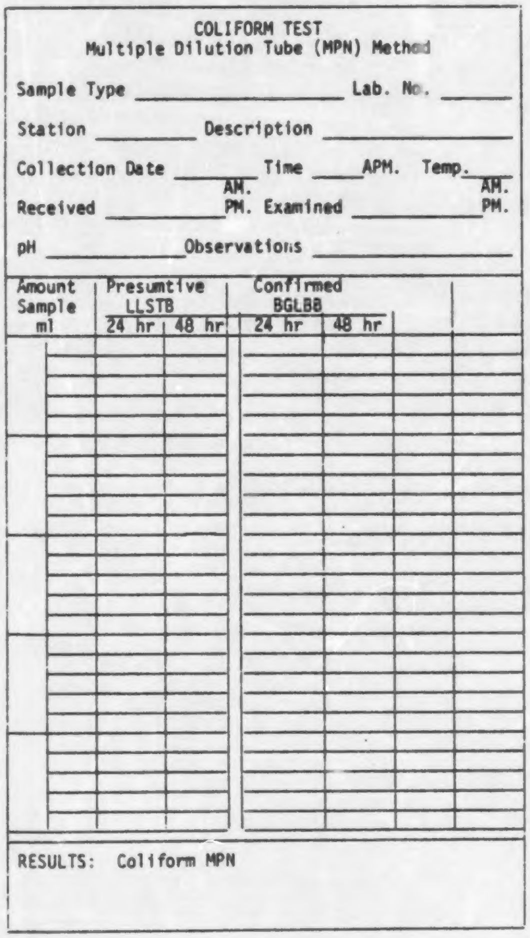
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.3.1	There is no such thing as a "standard" data sheet for bacteriological tests. A simplified data sheet is shown below.	
B.3.2	In this procedure, it is recommended that the worker learn to select a series of 4 sample volumes in decreasing amounts as indicated. It is possible to use as few as three sample volumes, but often the worker will fail to get a measurable result. On the other hand, one could have 5, 6, or even more sample volumes in decreasing amounts.	



COLIFORM TEST
Multiple Dilution Tube (MPN) Method

Sample Type __________ Lab. No. ______

Station ______ Description __________

Collection Date ______ Time ____ A.M. / P.M. Temp. ____

Received ______ A.M. / P.M. Examined ______ A.M. / P.M.

pH ______ Observations __________

Amount Sample ml	Presumtive LLSTB 24 hr		Confirmed BGLBB 24 hr	48 hr		
	24 hr	48 hr	24 hr	48 hr		

RESULTS: Coliform MPN

B.5.2 Suggested labeling code for tubes:

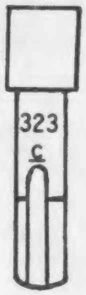
1. Every tube shows the laboratory bench number (323 in example shown below).

2. Below the laboratory bench number on each tube will be found a code symbol which represents the sample volume and the tube of each series of five. Thus:

Sample volume, ml	Tubes are labeled
10.0	A B C D E
1.0	a, b, c, d, e
0.1	$\underline{a}$, $\underline{b}$, $\underline{c}$, $\underline{d}$, $\underline{e}$
0.01	1a, 1b, 1c, 1d, 1e
0.001	2a, 2b, 2c, 2d, 2e
0.0001	3a, 3b, 3c, 3d, 3e
0.00001	4a, 4b, 4c, 4d, 4e
0.000001	5a, 5b, 5c, 5d, 5e
0.0000001	6a, 6b, 6c, 6d, 6e

etc., etc.

3. For example, a tube might look something like this, to represent sample No. 323, with the middle tube of a series of five representing 0.1 ml:



	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

B.6.

Multiple dilution tube tests for quantitative determinations apply a Most Probable Number (MPN) technique. In this procedure one or more measured portions of each of a series of decreasing sample volumes is inoculated into the first-stage culture medium. Through decreasing the sample increments, eventually a volume is reached where only one cell is introduced into some tubes. Each of the several tubes of sample-inoculated first-stage medium is tested independently, according to the principles described.

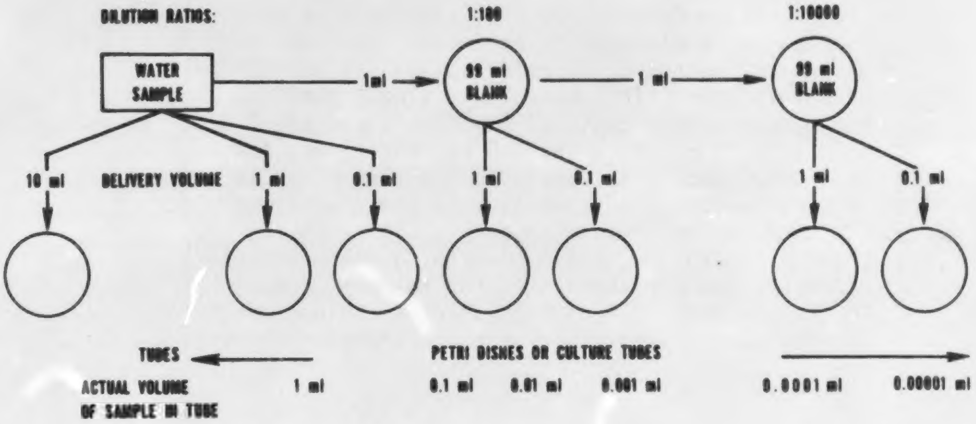
Another way to represent sample dilution and inoculation is shown below. Sample dilutions are made as needed during the inoculation procedure; they are not made up before starting to inoculate tubes of culture medium. Bacteria shall not be suspended in any dilution water for more than 30 minutes at room temperature.

Table of sample portions

To get (ml)		Deliver (ml)	From (sample preparations)
1.0		1.0	original sample
0.1	(1:10)	0.1	original sample
0.01	(1:100)	1.0	1:100 dilution
0.001	(1:1000)	0.1	1:100 dilution
0.0001	(1:10000)	1.0	1:10000 dilution
0.00001	(1:100000)	0.1	1:10000 dilution
0.000001	(1:1000000)	1.0	1:1000000 dilution

Dilutions of original samples

To get	Deliver to 99-ml blank	From
1:100	1 ml	Original sample
1:10000	1 ml	1:100 dilution
1:1000000	1 ml	1:10000 dilution


DILUTION RATIOS:
1:100
1:10000
WATER SAMPLE
1ml
99 ml BLANK
1 ml
99 ml BLANK
10 ml
DELIVERY VOLUME
1 ml
0.1 ml
1 ml
0.1 ml
1 ml
0.1 ml
TUBES
PETRI DISHES OR CULTURE TUBES
ACTUAL VOLUME OF SAMPLE IN TUBE
1 ml
0.1 ml
0.01 ml
0.001 ml
0.0001 ml
0.00001 ml

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.4.2	Transfers of LLSTB Transfers can be made, as indicated, with a wire loop having a diameter of at least 3 mm. An alternate method of transfer authorizes the use of an "applicator stick" which is a single service hardwood transfer device. Its dimensions are 0.2 to 0.3 cm in diameter and 2.5 cm longer than the test tube used in the analysis. The term single service denotes that the stick is pre-sterilized and used for a single transfer (LLSTB to BGLBB) and then discarded in the pan containing disinfectant and a new sterile stick used for the next tube to be transferred. Use of this stick technique makes the gas burner unnecessary for the transfer process	Std. Meth. 14:922

This outline was prepared by: Rocco Russomanno, Microbiologist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

COMPLETED TEST FOR THE MPN METHOD

as applied in

WATER TREATMENT FACILITIES
WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

BA.MET.lab.WMP.6.5.78

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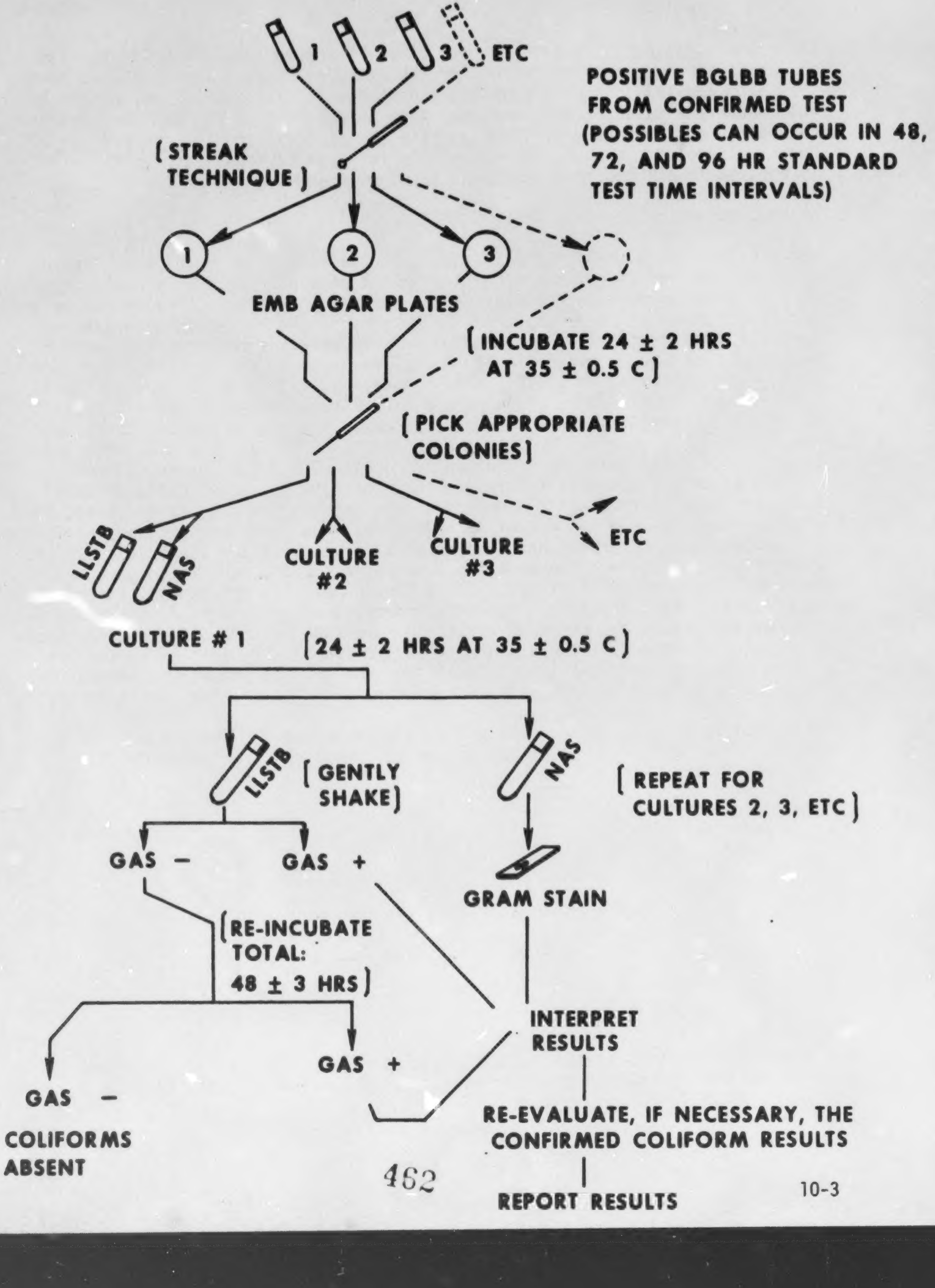
COMPLETED TEST SCHEMATIC


1
2
3
ETC
POSITIVE BGLBB TUBES FROM CONFIRMED TEST (POSSIBLES CAN OCCUR IN 48, 72, AND 96 HR STANDARD TEST TIME INTERVALS)
(STREAK TECHNIQUE)
1
2
3
EMB AGAR PLATES
(INCUBATE 24 ± 2 HRS AT 35 ± 0.5 C)
(PICK APPROPRIATE COLONIES)
LLSTB
NAS
CULTURE #2
CULTURE #3
ETC
CULTURE # 1
(24 ± 2 HRS AT 35 ± 0.5 C)
LLSTB
(GENTLY SHAKE)
NAS
(REPEAT FOR CULTURES 2, 3, ETC)
GAS −
GAS +
GRAM STAIN
(RE-INCUBATE TOTAL: 48 ± 3 HRS)
INTERPRET RESULTS
GAS +
GAS −
COLIFORMS ABSENT
RE-EVALUATE, IF NECESSARY, THE CONFIRMED COLIFORM RESULTS
REPORT RESULTS

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

1. Analysis Objectives:

In control testing, this test is part of the Standard Test for Coliforms and is one of the two tests of choice for reporting purposes. The completed test should be applied to such a proportion of tests as to establish beyond reasonable doubt the value of the confirmed test in determining the sanitary quality of water, and, as a rule of thumb, applied to at least ten (10) percent of all positive samples. When a specific sample is being tested, the completed test is applied to all positive confirmed tubes of that sample.

2. Brief Description of Analysis:

All positive tubes of BGLBB (Brilliant green lactose bile broth) from the confirmed test of the Standard Coliform MPN Test are individually and asceptically transferred onto EMB Agar by the streaking technique. After incubation for 24 ± 2 hours at 35 ± 0.5°C, one or more typical isolated colonies (dark-centered with or without sheen) or two or more atypical colonies (opaque; unnucleated; mucoid; or pink) if only these are present, are selected from each plate and transferred to LLSTB (lactose lauryl sulfate tryptose broth) and a nutrient agar slant (NAS). Each pure culture is incubated for 24 + 2 hours at 35 ± 0.5°C on these media and then inspected for gas formation (LLSTB) and growth (NAS). A gram stain is prepared from each NAS at this time with the slant asceptically (sterile technique) manipulated and then preserved under refrigeration for possible future need. A positive (gaseous) LLSTB is data recorded and discarded while a negative (non-gaseous) tube is re-incubated for an additional 24 hours (total of 48 ± 3 hours) when it is again inspected for gas production. Coliforms are considered to have populated the original BGLBB tubes if pure culture, gram-negative, non-spore forming rods which gaseously fermented lactose were isolated by this procedure. Any other results are considered to be the actions of non-coliforms except in the case of lactose fermentaters which are caused by mixed culture (two or more different organisms consisting of gram-positive and gram-negative forms). In this case, the retained nutrient agar slant is restreaked on EMB and the subsequent procedures repeated to attempt to isolate a pure culture with the coliform characteristics.

Adjustments, if any, are made to the tube codings and the MPN re-calculated to give a MPN completed result which is now the required reportable result.

This procedure conforms to the Standard Total Coliform MPN Test as described in Standard Methods for the Examination of Water and Wastewater, 14th Edition (1975), p. 916 ff.

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

Equipment and Supply Requirements

A. Capital Equipment:

1. *Incubator, air, to operate at 35° ± 0.5°C
2. *Oven, hot air, sterilizing-drying, to give uniform temperatures and with suitable thermometer to register accurately in range of 160-180°C
3. *Autoclave, providing uniform temperatures up to and including 121°C, equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperature within 30 minutes
4. Balance, 0.1 g sensitivity at load of 150 g
5. pH Meter, accurate to at least 0.1 pH unit, with standard pH reference solution(s)
6. Water distillation apparatus, (glass or block tin), or source of distilled water suitable for bacteriological operations
7. Microscope, compound, oil immersion lens, Abbé condenser

B. Reusable Supplies:

1. Apron or coat suitable for laboratory
2. Baskets, wire for discarded cultures
3. Tubes, culture*, 150 x 18 mm (metal caps for fermentation and screw-cap for slants)
4. Tubes, fermentation*, 75 x 10 mm vials to be inverted in culture tubes
5. Inoculation loop and needle, 3 mm diameter for loop and both of nichrome or platinum-iridium wire, 26 B&S gauge, in holders
6. Hotplate with magnetic whirl feature, if desired
7. Burner, gas, Bunsen burner type
8. Sponge, for cleaning desk top
9. Counter, colony, Quebec type, Darkfield Model with guide plate
10. Racks, culture type*, 10 x 5 openings, to accept tubes at least 25 mm in diameter
11. Pan, to receive discarded contaminated pipets and glassware (must contain disinfectant before use)
12. *Flasks, Erlenmeyer, 500 ml; 300 ml; 250 ml
13. *Cylinder, 500 ml; 250 ml

C. Consumable Supplies:

1. Bibulous paper
2. Dishes, petri, 100 x 15 mm sterile plastic, disposable
3. Disinfectant, for bench tops. (Can use household bleach solution prepared according to instructions on bottle.)
4. Distilled water, suitable for bacteriological cultures (Note distillation apparatus required in capital equipment.)
5. Eosin methylene blue agar, dehydrated (Levine modification)
6. Gram stain solutions, complete set
7. Lactose Lauryl Sulfate Tyrptose Broth, dehydrated
8. Nutrient agar, dehydrated
9. Slides, microscopic, glass 1" x 3"

10. Foil, aluminum
11. Matches or striker
12. Wax pencils (recommend soft as equivalent to Blaisdell 169T)

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*Items marked are needed in quantities or require size or space allowances which cannot be specified here, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specificatiors for equipment and supplies, see the Microbiology Section of the current edition of "Standard Methods for the Examination of Water and Wastewater."

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures		Aa. All pre-test procedures completed before starting other first-day proceudres.	
1. 35°C Incubator Set-up, Adjustment	1. Place 35°C incubator in permanent location.	1a. Out of drafts or places where it will be in sunlight part of day. 1b. Location convenient to laboratory bench 1c. Convenient source of electric power.	V.A.1 A.A.1.1 (p. 48)
	2. Install thermometer.	2a. Thermometer functions at least in 30°-40°C range and have intervals of 0.5° or less indicated. Meets NBS standards. 2b. Location should be central in incubator. 2c. Mercury bulb thermometer should be fitted with cork or rubber stopper and mounted in small bottle filled with liquid (glycerine, water, or mineral oil).	V.A.1.2 (p. 48)
	3. Install shallow pan of water in bottom of incubator.	3a. In most incubators a pan having about 1 square foot of area, with water about 1 inch deep, is satisfactory. 3b. Maintains condition of saturated relative humidity, required in bacteriological incubator. 3c. Requires daily check, with addition of water as necessary, to keep water in pan at all times.	V.A.1.3 (p. 48)
	4. Connect incubator to electric power source.	4a. Many incubators have pilot light to indicate power turned on.	
	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for method temperature adjustment. 5b. Operation must be at 35° ± 0.5°C. 5c. Allow about 1 hour between adjustments.	V.A.1.5 (p. 48)
	6. Operate bacteriological incubator continuously.	6a. Requires daily check with written temperature record, with adjustment and water addition as necessary.	V.A.1.6 (p. 48)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)			
2. Oven, Sterilizer-Dryer Set-up	1. Place oven sterilizer in permanent location.	1a. Convenient to source of electric power usually on table or bench.	V.A.2.1-5 (p. 49)
	2. Install thermometer.	2a. Should indicate the 160°-180° range, be accurate within this interval, and be marked in 1.0 degree intervals.	
	3. Connect oven sterilizer to power source and turn on.	3a. Usually has pilot light to indicate power on.	
	4. Adjust temperature to stabilize at required temperature.	4a. Operated as near to 170°C as possible; not lower than 160° or higher than 180°C.	
	5. Operate oven sterilizer only when needed. Turn off when not in use.	5a. Unless materials are "heat shocked" adversely, oven is turned ON in advance of need to permit reaching required temperature before introducing material. 5b. Oven used to sterilize or dry glassware, metal objects. 5c. Oven sterilizer not used with culture media, solutions, plastics, rubber objects, or with anything containing or including these. 5d. Paper-wrapped glass pipets, graduates, flasks, etc. may be sterilized in oven sterilizer.	
3. Autoclave Set-up	1. Install and operate autoclave according to manufacturer's instructions.	1a. Autoclaves extremely variable in design and operation; also, potentially dangerous. 1b. Used to sterilize objects made of, or including liquids, rubber, culture media. 1c. Glassware may be autoclave sterilized but must be dried afterward. 1d. Most plastics not sterilized in autoclave; plastics usually require chemical sterilizers. 1e. Autoclave usually operated at 121°C for 15 minutes. 1f. Sterilized media must be removed from autoclave as soon as possible after autoclave is reopened.	V.A.3.1 (p. 49)

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)			
4. Water Distillation Equipment	1. Install and operate in accordance with manufacturer's instructions.	1a. Must produce distilled water meeting quality requirements for bacteriological tests.	V.A.4.1-2 (p. 49)
	2. Operate as required to maintain adequate supplies of distilled water.	2a. Reserve supplies kept in borosilicate glass carboys or in plastic carboys made of material which will not dissolve substances which will affect growth of bacteria. 2b. Same distillation apparatus used for bacteriological purposes may be used for chemical reagents.	
5. pH Meter	1. Have unit available and operable.	1a. Unit for pH check on finished culture media.	V.A.5.1 (p. 50)
6. Glassware	1. Wash all glassware in hot detergent solution.	1a. Nontoxic detergent. 1b. Be sure all contents and markings are washed away.	V.A.6.1-4a (p. 50)
	2. Rinse at least once in hot tap water.		
	3. Rinse in distilled water, at least 6 successive times and,		
	4. Dry in air or oven.	4a. No visible spots or scum; glass should be clean and sparkling. 4b. Glassware suitable for use in bacteriological operations.	V.A.6.4b (p. 50)
7. Preparation of Lactose Lauryl Sulfate Tryptose Fermentation Broth (LLSTB)	1. Weigh 8.9 grams of dehydrated Lactose Lauryl Sulfate Tryptose Broth. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media takes moisture out of air; can become caked. 1b. Caked media unsatisfactory; should be discarded.	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	2. Dissolve in 250 ml distilled water.	2a. Use a 500 ml Erlenmeyer flask. 2b. Gentle heat (no boiling) if necessary to complete dissolving medium. Usually a vigorous agitation will completely dissolve the medium.	
	3. Place 10.5 ml of the solution of prepared LLSTB in each culture tube.	3a. Use 150 x 18 mm tubes. 3b. A 25 ml pipet, automatic pipetter, or funnel, hose and pinchcock assembly are acceptable. 3c. Accuracy of delivery: ± 0.5 ml. 3d. Approximately 23 tubes will be necessary.	V.A.7.3 (p. 50)
	4. Insert one fermentation vial into each tube of medium, open end down.	4a. Tubes and vials washed as indicated previously. 4b. Use 75 x 10 mm tubes.	
	5. Place tube cap on each tube of culture medium.	5a. After all tubes have been filled and have individual vial.	
	6. Sterilize in autoclave.	6a. Within 1 hour after medium prepared. 6b. Sterilization at 121°C for 15 minutes. 6c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" mode of steam removal.	
	7. Cool medium to room temperature.	7a. Medium ready for use when cool and individual vials are completely filled with fluid. No bubbles must be present. Wait for complete cooling before checking for bubbles.	
	8. Check pH of finished medium.	8a. Should be pH 6.7-6.9. It is rare that deviations occur with this preparation.	
	9. If final pH not satisfactory, discard medium and prepare new batch with pH adjustment before sterilization.	9a. pH value ordinarily drops about 0.2 pH unit. 9b. Check for dirty glassware, acid residues in glassware, etc.	

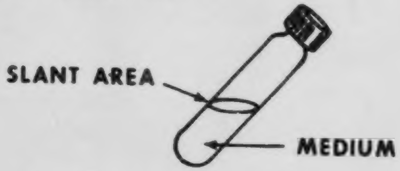
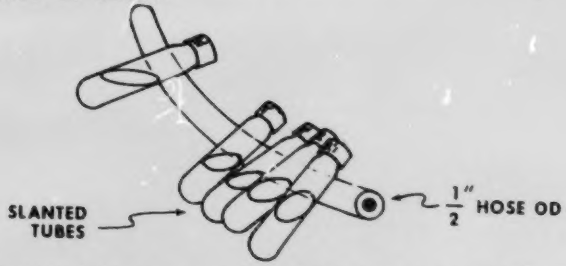
WATER MONITORING PROCEDURE: Completed Test for the MPN Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	10. Store medium in cool, dark place.	10a. Not in refrigerator. Usually in laboratory cabinet in darkness. 10b. May be stored up to 1 week if evaporation not more than 10% in loose-fitting capped tubes. With screw-capped tubes, it should be held no longer than 3 months.	
8. Preparation of Eosin Methylene Blue Agar (EMB Agar)	1. Weigh 7.5 grams of dehydrated eosin methylene blue agar. Close cover of bottle of dehydrated medium tightly after removal.	1a. Use only Levine's Modification as this medium has a number of modifications for differing purposes. 1b. Dehydrated media takes moisture out of air; can become unacceptably caked.	
	2. Dissolve in 200 ml distilled water.	2a. Use a 300 ml Erlenmeyer flask with double layer foil cap. 2b. Heat to boiling to dissolve completely. Do not prolong boiling. 2c. Frequent agitation is necessary to prevent burning of medium. 2d. All of the agar must be in solution. Agar will be recognized as particulate matter along the sides of the flask. Gently swirl flask until all of this material is off of sides and into medium.	V.A.8.2c (p. 50)
	3. Sterilize medium in autoclave.	3a. For 15/15 to effect complete sterilization (15 psi for 15 minutes). 3b. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" mode of steam removal.	
	4. Cool medium to 50-60°C and pour into sterile petri dishes.	4a. Can also be poured "hot" from autoclave with precautions, such as using asbestos glove, for personal protection.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Proceudres (Continued)		4b. A flocculant may form after autoclaving. Swirl flask gently during plate (dish) filling. 4c. About 10-12 mls/plate. About 15 plates will be required. 4d. Cover plates as they are poured. Do not place covers on bench where they can become contaminated	
	5. Allow dishes to cool to room temperature and then dry.	5a. Agar will solidify and allow plate to be moved without disturbing medium. 5b. Invert plates (turn upside down) and place in 35° incubator overnight. This will allow plates to dry and remove excess moisture. 5c. Plates can be used when agar surface is "dry" (does not have water droplets).	
	6. Check pH of one of the plates.	6a. Insert pH meter probes into the agar medium using one of the plates of the batch. 6b. Should read 7.0-7.2. 6c. Discard plate after measuring pH. Alternately, to save medium, one could fill a small clean receptacle, or, a 60 x 15 mm petri dish for this check. 6d. Out of range reading denotes unacceptable procedure, equipment or materials used (dirty glassware, poor water supply, overheating, etc.). Discard plates and rectify problem.	
	7. Label and date batch of plates. Store either at room temperature when use is made of plates within several days or in sealed plastic bags, at 4°C.	7a. Can be kept for one month under refrigeration as described. Plates may have to be re-dried in the incubator overnight (inverted) after removal from refrigerator.	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)			
9. Prepare Gram-Stain Solutions	1. Prepare solutions as recommended by manufacturer.	1a. Premixed dyes will probably only require dilution. 1b. If desirous to prepare dyes from scratch, consult Standard Methods for procedure.	Std. Meth. 14:918-919
	2. Place in dropper bottles for use.		
10. Prepare Nutrient Agar Slants (NAS)	1. Weigh 2.9 grams of dehydrated Nutrient Agar. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media takes moisture out of air; can become caked. 1b. Caked media unsatisfactory; should be discarded.	
	2. Dissolve in 125 ml distilled water.	2a. Use a 250 ml Erlenmeyer flask with double layer foil cap. 2b. Heat to boiling to completely dissolve. 2c. Frequent agitation is necessary to prevent burning of medium. 2d. All of agar must be in solution. Agar will be recognized as particulate matter along the sides of the flask. Gently swirl flask until all of this material is off of sides and into medium.	See V.A.8.2c (p. 50)
	3. Dispense 6-7 mls of medium into screw-cap tubes.	3a. Use 150 x 18 mm screw-cap tubes. 3b. A 10 ml pipet, automatic pipetter, or funnel, hose, and pinchcock assembly are acceptable. 3c. Approximately 25 tubes will be required.	See V.A.7.3 (p. 50)
	4. Place screw caps loosely on each tube which are packed loosely in a test tube rack, beaker, etc.	4a. Allows steam to penetrate to medium.	
	5. Sterilize tubes in autoclave.	5a. For 15/15 to effect complete sterilization (15 psi for 15 minutes). Remove medium as soon as possible after cycle (slow vent mode) is completed.	See V.A.3.1 (p. 49)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued)	6. Tighten caps and slant hot medium.	6a. Tight caps will prevent further loosening and possible contamination. 6b. Necessary to slant while hot so that medium will not solidify in upright position. 6c. "Slanting" is done to allow a large surface area for growth of bacteria.  6d. Apparatus for tube holding while in the slanted position can range from expensive "angle" controlled supports to as simple and effective a method as below: 	
	7. Allow tubes to solidify before removing from slanted position and placing in test tube rack.	7a. Solidified tubes can be picked up and will retain "slanted" position of medium. Tubes will start to harden below 40°C and take on an "opaque" form as they harden.	
	8. Date and label medium as Nutrient Agar. Store in refrigerator.	8a. Temperature 1-4.4°C. 8b. Can be stored for up to 3 months (if kept in dark and evaporation is not excessive (less than .25 ml).	

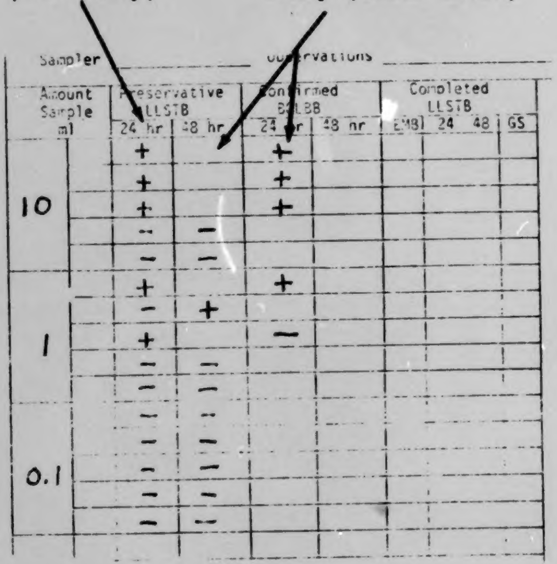
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures (Continued) 11. Final Equipment and Supply Check	1. Check to be sure that all equipment and supplies, solutions, and prepared media are ready before starting sample examination.	1a. Check general list of equipment and supplies. 1b. Each test requires: 1-15 EMB agar plates 1-20 Nutrient Agar Slants 1-20 LLSTB tubes 1 Bacteriological loop 1 Bacteriological needle 1-20 Microbiological slides Gram stain reagents, set Since, as shown, the numbers of items can vary (depending upon the number of confirmed test positives and subsequent EMB colony forms) this WMP (Water Monitoring Procedure) will specifically pick a hypothetical situation which will give the reader a cross-section of conditions which could occur.	
B. Initial Procedures 1. Equipment Maintenance	1. Check, record, and adjust incubator temperature.	1a. See A.1.1-6. 1b. Should be in operating condition since MPN test's earlier phases are in progress (presumptive and confirmed tests).	
	2. Add water to pan in incubator as necessary.		
2. Data Sheet Inspection	1. Locate data sheet and verify that the required sample is being processed.	1a. A "new" data sheet does not have to be initiated since the sample is already being processed.	VII.B.2 (Suggested Data Sheet) (p. 52)

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued)	2. Use active sheet of the 48 hour MPN test (partial completion of the confirmed test) with 48 hour presumptive tubes and 24 hour confirmed tubes "saved."	2a. For our hypothetical test, the data sheet shows as follows:	

24 hour column entry (tubes processed previously)

48 hour column entry (tubes saved)

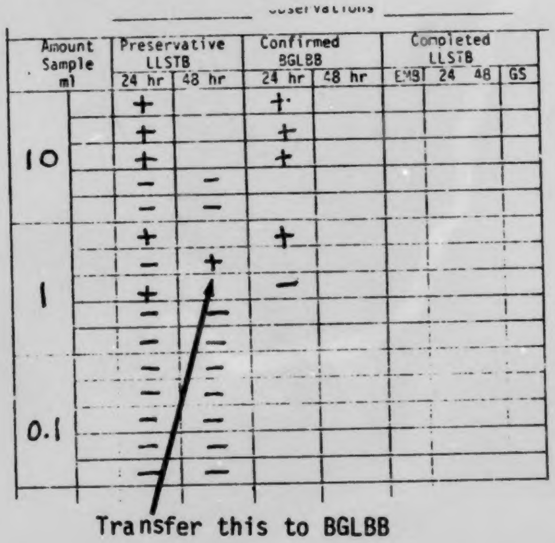


Sampler ______ Observations ______

Amount Sample ml	Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24	Completed LLSTB 48	GS
10	+		+					
	+		+					
	+		+					
	−	−						
	−	−						
1	+		+					
	−	+						
	+		−					
	−	−						
	−	−						
0.1	−	−						
	−	−						
	−	−						
	−	−						
	−	−						

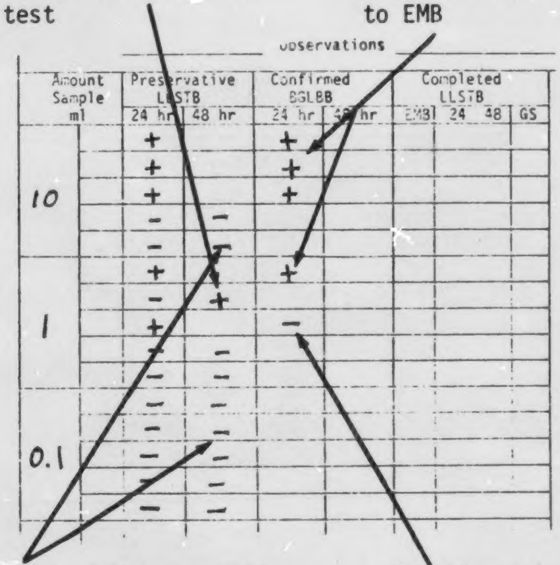
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
		2b. Tubes "saved" will be used to initiate the completed test or to proceed to the confirmed test.	
3. Lab Bench Disinfection	1. Disinfect laboratory bench; wipe dry.	1a. Sponge and disinfectant; paper toweling.	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued) 4. Continue Standard MPN Test Procedure	1. Transfer positive LLSTB tubes of the presumptive stage.	1a. From data sheet (B.2.2.2a), note that one tube will be transferred from the presumptive stage to the confirmed stage:  Transfer this to BGLBB 1b. Progress of this transfer will be monitored for possible inclusion to the comleted test.	Std. Meth. 14:917

Observations

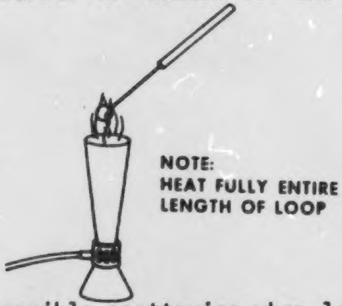
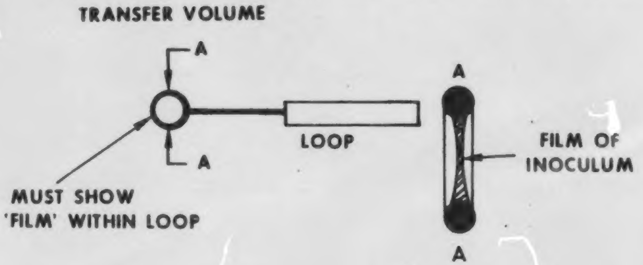
Amount Sample ml		Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24	Completed LLSTB 48	GS
10		+		+					
		+		+					
		+		+					
		−	−						
		−	−						
1		+		+					
		−	+						
		+		−					
		−	−						
		−	−						
0.1		−	−						
		−	−						
		−	−						
		−	−						
		−	−						

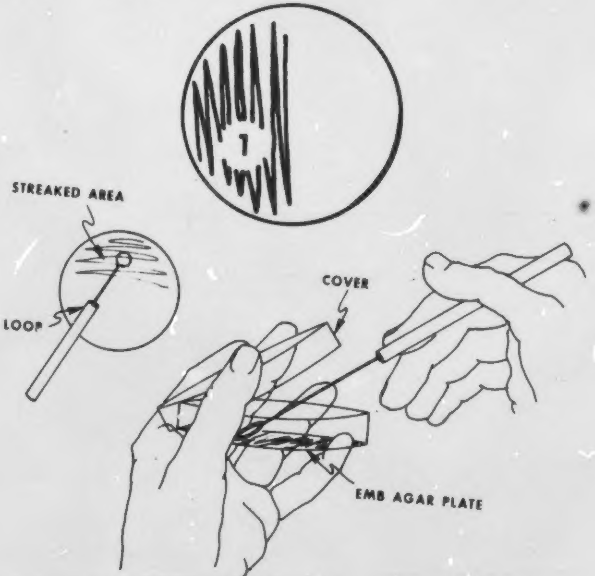
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued) Confirmed Test Start First Day Procedures 5. Select BGLBB Positives from Confirmed Test	1. Select "positives" from confirmed "24" hour tubes for processing.	1a. Four positive BGLBB tubes are to be processed:  This positive transferred to confirmed test Process these 4 tubes to EMB Discard these negatives... coliforms absent (see schematic) Negative tube to be re-incubated as per confirmed test requirements (Std. Meth. 14:920)	

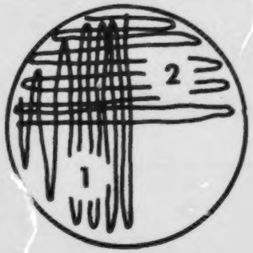
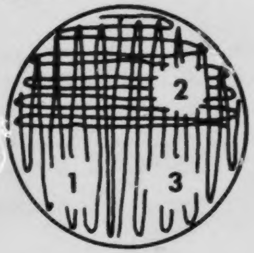
Observations

Amount Sample ml	Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB EMB	Completed LLSTB 24	Completed LLSTB 48	GS
10	+		+					
	+		+					
	+		+					
	−	−						
	−	−						
1	+		+					
	−	+						
	+		−					
	−	−						
	−	−						
0.1	−	−						
	−	−						
	−	−						
	−	−						
	−	−						

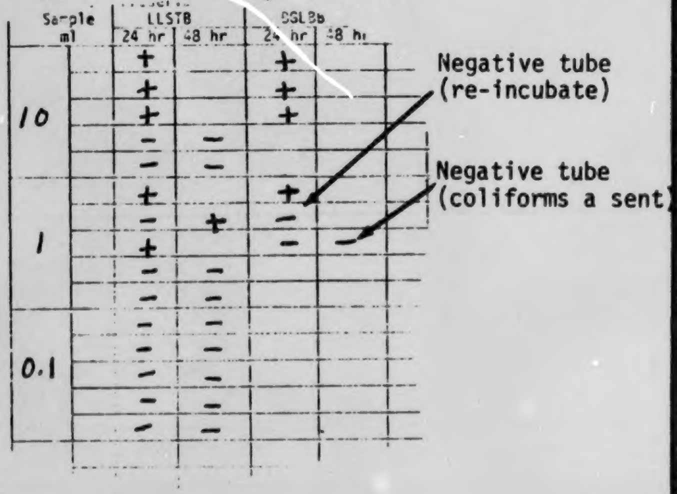
WATER MONITORING PROCEDURE: Completed Test for the MPN Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued) 6. Prepare EMB Agar Plates	1. Shake all positive BGLBB tubes vigorously after labeling each tube.	1a. Labeling avoids correlation errors in tube plate matching. Mark tubes 10/1; 10/2; 10/3; and 1/1 for the four positive tubes in order (labeled according to inoculation volume/number in row). 1b. Shaking allows organisms to be suspended in the broth.	
	2. Sterilize a bacteriological loop.	2a. Heat in burner to redness all the way to handle: 	
	3. Allow loop to cool (5-10 seconds).	3a. Avoids possible spattering when loop is inserted into tube.	
	4. Remove cap from the first positive BGLBB tube (10/1).		
	5. Insert loop into broth to obtain film transfer. Cover tube and discard.	5a. "Film" within loop represents transfer volume.  TRANSFER VOLUME A A LOOP A FILM OF INOCULUM A MUST SHOW 'FILM' WITHIN LOOP	V.B.6.5 (p. 50)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued)	6. Streak transfer inoculation from loop to corner of EMB agar plate.	6a. Agar surface must be dry for satisfactory results. 6b. Streak the inoculation lightly back and forth over half the agar surface, as in ①, avoiding scratching or breaking the agar surface.  6c. Use asceptic (sterile) technique to prevent contamination of medium. Close cover of petri dish when not streaking.	VII.B.6.6 (p. 52)
	7. Sterilize (flame) loop and air-cool as before.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued)	8. Streak another segment of plate to carry portion of inoculation into another area of medium.	8a. Turn Petri dish about one-quarter turn in the holding hand (allows easier streaking). 8b. Streak the loops, tip lightly back and forth over one-half the agar surface, working from area ① into one-half the unstreaked area of the agar.  8c. Technique allows "dilution" of original heavy inoculum to occur into an area where less growth will now result.	
	9. Sterilize loop and air cool.		
	10. Streak the remaining unstreaked area of medium.	10a. Turn the Petri dish one-quarter turn in the holding hand. 10b. Streak the tip lightly back and forth over one-half the agar surface, working from area 2 into area ③. 	

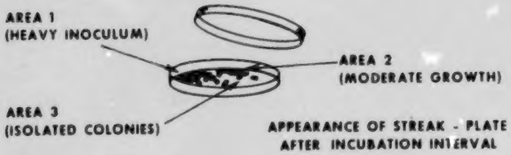
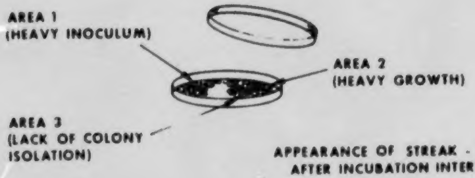
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Initial Procedures (Continued)		10c. Do not allow any of streaks of one group to touch a more concentrated area than the area streaking from (in effect, separate 3 from 1 or over-growth may occur.) 10d. Close the culture container, and, until the colonies (bacterial growth forms) are picked, keep the top and bottom as a unit without allowing separation to occur.	
	11. Flame sterilize the loop and set it aside.		
	12. Invert dish (turn up-side-down) and identify.	12a. Use grease pencil (wax pencil) to label bottom of dish. 12b. For this positive BGLBB tube label as follows: 217 — Lab number for this particular sample 10/1 — Indicates 1st tube of row which received 10 ml sample inoculation	
	13. Incubate EMB agar plate.	13a. At 35° ± 0.5°C for 24 hours. 13b. Keep in inverted position (avoids water droplets, if formed, from falling on the medium surface and ruining the plate).	
	14. Streak, label, and incubate EMB plates from the other three positive BGLBB tubes (10/2; 10/3; and 1/1).	14a. Use techniques for streaking as previously described. 14b. Labeled plates will read: 217 10/2 217 10/3 217 1/1 14c. Incubate as previously described.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedure 1. Equipment Maintenance	1. Check, record, and adjust incubator temperature.		
2. Lab Bench Disinfection	1. Disinfect laboratory bench, wipe dry.	1a. Sponge and disinfectant; paper toweling.	
3. Data Sheet Recordings	1. Locate required data sheet.	1a. Sample "217" in our example.	
	2. Remove cultures from incubator and assemble with data sheet.	2a. 4 EMB plates (24 hours old) 1 BGLBB tube (24 hours old) 1 BGLBB tube (48 ± 3 hours old)	See B.2.2a
	3. Read BGLBB tubes for gas and record results.	3a. Any amount of gas is considered positive. Shake tubes gently before reading. 3b. Assume the following results: 	See Schematic Diagram (p. 3)

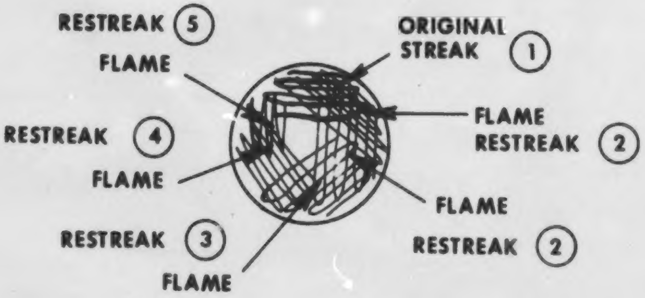
Sample ml	LLSTB 24 hr	LLSTB 48 hr	BGLBb 24 hr	BGLBb 48 hr
10	+		+	
	+		+	
	+		+	
	−	−		
	−	−		
1	+		+	
	−	+	−	
	+		−	−
	−	−		
	−	−		
0.1	−	−		
	−	−		
	−	−		
	−	−		
	−	−		

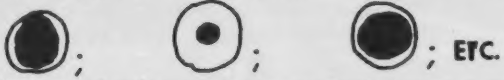
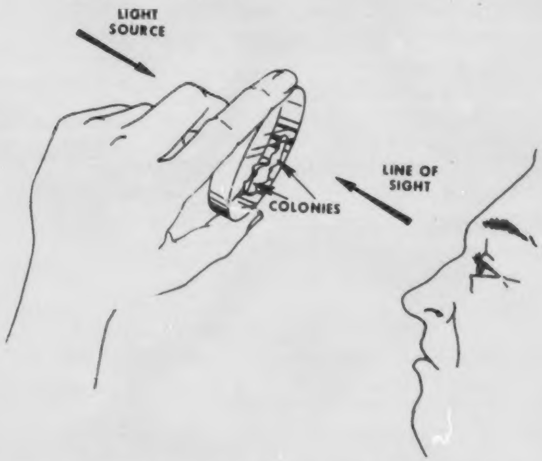
Negative tube (re-incubate)

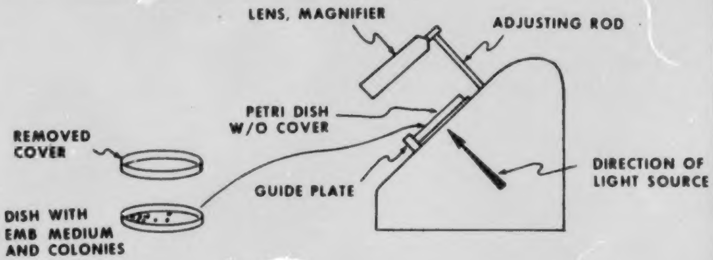
Negative tube (coliforms a sent)

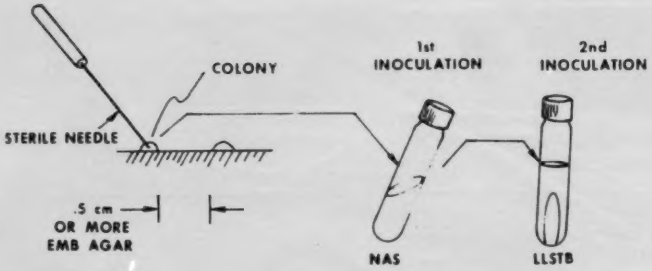
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedure (Continued)	4. Discard any BGLBB tubes which are negative in 48 hours.	4a. There is one such tube (1 ml sample volume; 3rd tube of row). 4b. Coliforms absent in this tube.	
	5. Save any 24 hour BGLBB tube which is positive or negative.	5a. None are positive. This possibility would have made it necessary to streak an EMB agar plate. 5b. There is a negative. Re-incubate this for an additional 24 hours (1 ml sample volume; 2nd tube of row).	
4. EMB Agar Plate Inspection	1. Remove cover from one of the four EMB agar plates and inspect growth (most convenient to open plate 10/1).	1a. Usual plate growth (colonies) will be as indicated:  AREA 1 (HEAVY INOCULUM) AREA 2 (MODERATE GROWTH) AREA 3 (ISOLATED COLONIES) APPEARANCE OF STREAK - PLATE AFTER INCUBATION INTERVAL OR OCCASIONALLY,  AREA 1 (HEAVY INOCULUM) AREA 2 (HEAVY GROWTH) AREA 3 (LACK OF COLONY ISOLATION) APPEARANCE OF STREAK - PLATE AFTER INCUBATION INTERVAL	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedure (Continued)		1b. In the case of isolated colonies, one could proceed to the next step of the completed test (C.5). 1c. In the case of a lack of isolated colonies, one must proceed to re-streak another plate to attempt isolation of a colony. (As follows): Re-isolation Procedure A. Flame sterilize a loop and air cool B. Immerse the loop into an area which shows a representative growth mass. Occasionally, the loop must be touched to two or three masses to obtain this material. C. Close cover and discard EMB plate. D. Streak plate of fresh, sterile, dry EMB agar using the same technique as previously outlined except that it would be wise to allow more streaking sequences with an increased number of loop flamings. This would more likely ensure better isolation:  RESTREAK ⑤ ORIGINAL STREAK ① FLAME FLAME RESTREAK ② RESTREAK ④ FLAME FLAME RESTREAK ③ RESTREAK ② FLAME E. Incubate as previously outlined.	III.C.4.1 (p. 47)

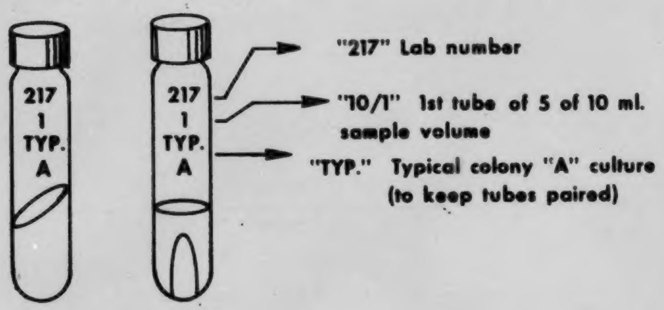
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedure (Continued)		1d. Pure growths (colonies) can be regarded as falling into two groupings: I. Typical Colonies (characteristic of coliforms) Colonies with dark centers commonly termed "nucleated" or "fisheye" when viewed from the bottom of the plate:  These colonies may or may not have a metallic-like sheen characteristic on the surface of the colony.  II. Atypical Colonies (usually a non-coliform) These colonies may be opaque, unnucleated, mucoid, or pink after the prescribed incubation period.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedure (Continued)			
5. EMB Agar Plate Colony Transfer	1. Transfer pure cultures to LLSTB and NAS.	1a. Use flamed and air cooled needle for fishing (picking). 1b. Use of colony counter as a magnification aid is recommended: 	
		1c. Pick one or more typical colonies, or, two or more atypical colonies and transfer each of them into their own set of tubes (LLSTB and NAS)	(See schematic of test)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedure (Continued)		1d. Recommended technique is to pick a pure colony and, with a single transference, inoculate both the LLSTB and the NAS in a single motion.	
		 COLONY 1st INOCULATION 2nd INOCULATION STERILE NEEDLE .5 cm OR MORE EMB AGAR NAS LLSTB	
		NAS...Flame top of tube for about two seconds prior to entering with needle. Gently swab surface of agar medium. Replace screw-cap which is held in hand without contaminating during procedure.	
		EMB...Discard plate after inoculations.	
		LLSTB...Transfer inoculum directly to LLSTB tube. (Return to colony is not necessary.) Flaming of tube top not necessary. Shake needle in broth for transfer.	

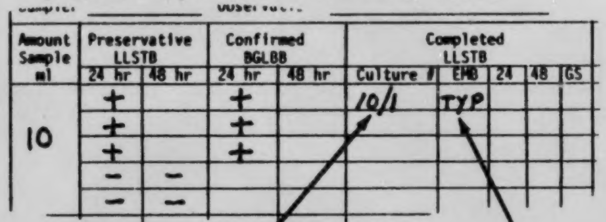
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedure (Continued)			

1e. Label tubes for identification. Such a labeling could be as follows:


217
1
TYP.
A
217
1
TYP.
A
"217" Lab number
"10/1" 1st tube of 5 of 10 ml. sample volume
"TYP." Typical colony "A" culture (to keep tubes paired)

1f. Incubate tubes at 35° ± 0.5°C.

1g. Indicate the necessary information regarding the step just completed on the data sheet:



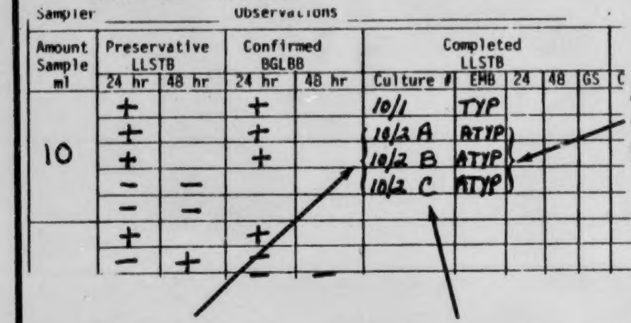
Amount Sample ml	Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB Culture #	EMB	24	48	GS
10	+		+		10/1	TYP			
	+		+						
	+		+						
	−	−							
	−	−							

1st tube of row of 10 ml sample volume

EMB culture was "typical" (See C4.1.1d for definition)

NOTE: Only one colony will be processed for this first tube of the first row since the colony is typical (one or more could have been picked).

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedure (Continued)		1h. Utilizing the same procedures as in a - g of this section, process the next culture (10/2 EMB agar plate). Assuming an atypical colony formation on the plate, we will process three cultures (2 or more are required for this condition) and record them as follows:	



Sampler __________ Observations __________

Amount Sample ml	Preservative LLSTB		Confirmed BGLBB		Completed LLSTB					
	24 hr	48 hr	24 hr	48 hr	Culture #	EMB	24	48	GS	C
10	+		+		10/1	TYP				
	+		+		10/2 A	ATYP				
	+		+		10/2 B	ATYP				
	−	−			10/2 C	ATYP				
	−	−								
	+		+							
	−	+	−	−						

Atypical colonies on EMB

Three cultures processed

Letter designations will keep the three cultures separated (Note: the same "A"; "B" and "C" will appear on the LLSTB tubes and NAS tubes.)

1i. Process the two remaining cultures and assume the following recordings for all four cultures:

(See data sheet on following page.)

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

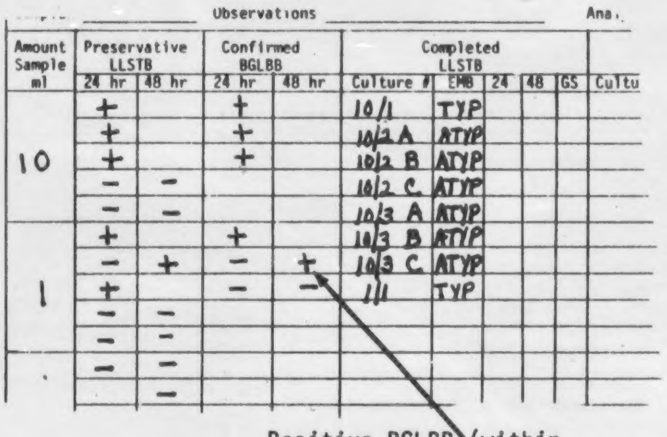
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second-Day Procedure (Continued)			

________ Observations ________________ Analyst ________

Preservative LLSTB		Confirmed BGLBB		Completed LLSTB					Completed LLSTB			
24 hr	48 hr	24 hr	48 hr	Culture #	EMB	24	48	GS	Culture #	EMB	24	48
+		+		10/1	TYP							
+		+		10/2 A	ATYP							
+		+		10/2 B	ATYP							
−	−			10/2 C	ATYP							
−	−			10/3 A	ATYP							
+		+		10/3 B	ATYP							
−	+	−		10/3 C	ATYP							
+		−	−	1/1	TYP							
−	−											
−	−											
−	−											
−	−											
−	−											
−	−											
−	−											

D. Third-Day Procedure 1. Equipment Maintenance	1. Check, record, and adjust incubator temperature.		
2. Lab Bench Disinfection	1. Disinfect laboratory bench.	1a. Sponge and disinfectant; paper toweling.	
	DATA SHEET ENTRIES		
3. Test Observations, Recordings, and Processing	1. Locate required data sheet.	1a. Sample "217" is our example.	
	2. Remove cultures from incubator and assemble with data sheet.	2a. 8 NAS, 8 LLSTB } 24 hours incubation	
		1 BGLBB 48 ± 3 hours of incubation	See C.3.3b

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedure (Continued)	3. Read BGLBB tube for gas and record results.	3a. Tube labeled $\frac{217}{1/2}$ after we find it is positive (any amount of gas considered positive).	



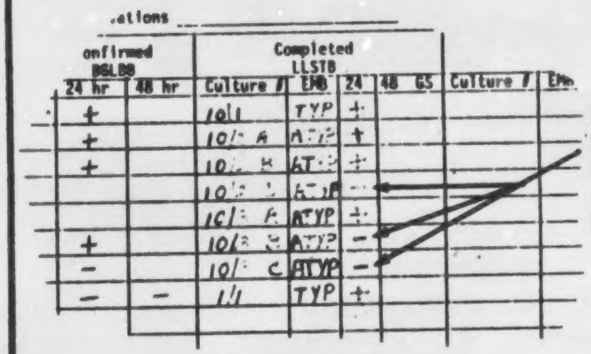
Observations Ana.

Amount Sample ml	Preservative LLSTB 24 hr	Preservative LLSTB 48 hr	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB Culture #	Completed LLSTB EMB	24	48	GS	Cultu
10	+		+		10/1	TYP				
	+		+		10/2 A	ATYP				
	+		+		10/2 B	ATYP				
	−	−			10/2 C	ATYP				
	−	−			10/3 A	ATYP				
1	+		+		10/3 B	ATYP				
	−	+	−	+	10/3 C	ATYP				
	+		−	−	1/1	TYP				
	−	−								
	−	−								
	−	−								
		−								

Positive BGLBB (within 48 ± 3 hours)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
		3b. Tube would have been discarded if it were negative (coliforms absent) and a negative (-) in this case, assigning a tube number would have been unnecessary.	
		3c. Process this (+) BGLBB tube to an EMB agar streak plate as outlined previously and then discard the (+) tube.	See B.6

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedure (Continued)	4. Check NAS and LLSTB tubes for growth and gas, respectively and record results.	4a. Eight pairs of tubes to be read and recorded. Assume the following:	



.ations

onfirmed BGLBB		Completed LLSTB						
24 hr	48 hr	Culture #	EMB	24	48	GS	Culture #	EMB
+		10/1	TYP	+				
+		10/[illegible] A	ATYP	+				
+		10/[illegible] B	ATYP	+				
		10/[illegible]	ATYP	−				
		10/[illegible] A	ATYP	+				
+		10/[illegible] B	ATYP	−				
−		10/[illegible] C	ATYP	−				
−	−	1/1	TYP	+				

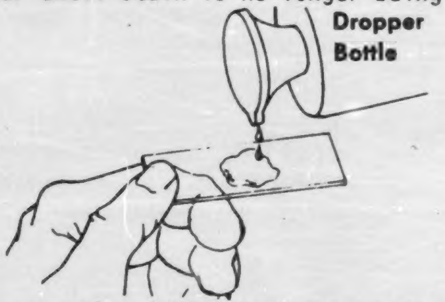
Re-incubate negatives for an additional 24 hours.

4b. Growth on the NAS is readily visible as an opaque mass which was not present on the sterile medium. No recordings are necessary for this growth--it will be used for a GRAM STAIN. It is quite rare that no growth will occur on this medium. (If this rarity occurs, restreak the NAS tube from its companion LLSTB tube.)

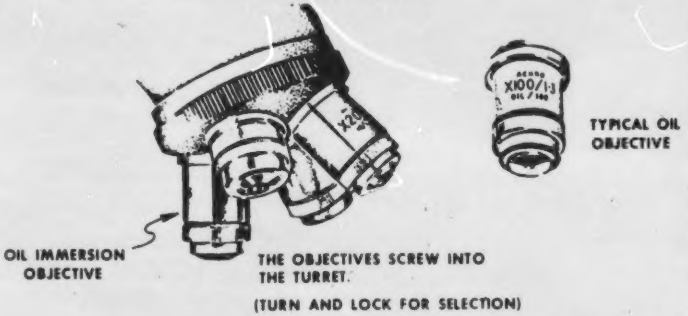
WATER MONITORING PROCEDURE: Completed Test for the MPN Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedure (Continued)	GRAM STAINING		
	5. Assemble Gram staining materials and cultures.	5a. 8 bacteriological glass slides 1 dropper bottle containing ammonium oxalate crystal violet dye 1 dropper bottle containing Lugols solution (Grams modification) 1 dropper bottle containing safranin dye 1 dropper bottle containing acetone-alcohol 1 squeeze bottle containing tap water bibulous paper 8 NAS cultures...(24 hour cultures) 10/1 10/3 A 10/2 A 10/3 B 10/2 B 10/3 C 10/2 C 1/1	Std. Meth. 14:918-19 III.D.3.5 (p. 47) VII.D.3.5 (p. 53)
	6. Prepare glass slides.	6a. Must be clean. 6b. Helpful to clean with alcohol, distilled water, and lens tissue. 6c. Place a drop of distilled water about 1 inch from end of slide.	
	7. Place NAS culture on slide (use 10/1 culture first then repeat procedure with 10/2 A, 10/2 B, etc., each on a different slide).	7a. Screw-cap tube handled asceptically (sterile technique): * flame top of tube * needle flamed to sterilize * cap handled carefully and returned promptly to tube 7b. NAS stored in refrigerator for possible need. 7c. Only minute amount of culture necessary. Large amounts can cause staining problems. 7d. Place culture from needle with water droplet on slide and mix well while extending the droplet size to about a 1" x 1/2" area.	

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

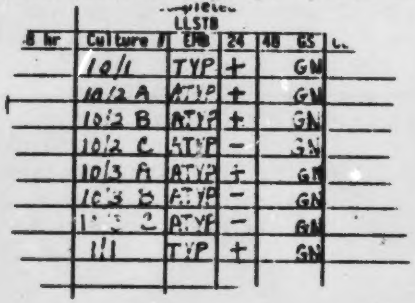
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedure (Continued)	8. Prepare culture for staining procedure.	8a. Allow smear to air-dry completely and then heat fix by by-passing slide (culture on upper side) through the gas flame briefly back-and-forth for a heat exposure of about two seconds.	
	9. Stain culture with reagents on the side of the slide with the culture.	9a. Flood the slide with ammonium oxalate-crystal violet dye. 9b. Allow to cover culture area for 1 minute. 9c. Wash slide gently with tap water. 9d. Apply Lugols-iodine solution to culture area. 9e. Allow to cover culture area for 1 minute. 9f. Wash slide gently with tap water. 9g. Apply acetone-alcohol solution to culture area. Hold slide and allow solution to flow across smear until stain is no longer being removed:  9h. Do not prolong this alcohol contact period (decolorization step) as the results may be erroneous. Some authorities suggest 10-15 seconds maximum. 9i. Wash slide gently with tap water. 9j. Apply Safranin solution (counter-stain) for 15 seconds and then wash gently with tap water. 9k. Blot slide gently with bibulous paper using care not to rub culture area during procedure.	Std. Meth. 14:918-19

(Continued on next page)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedure (Continued)		9l. Identify slide to conform to proper culture being examined. Use of a slide label is convenient (label "217 10/1" as per our example).	
	10. Repeat step sequences 7-9 for cultures 10/2 A; 10/2 B; 10/3 A; and 1/1.		
	11. Examine slides microscopically.	11a. If desired, slides can be retained for later examination. If the lactose (LLSTB) broth remains negative for the culture (48 ± 3 hours), the slide need not be examined as the culture is NOT a coliform.	
		11b. Become acquainted with microscope from manufacturer's literature or individual acquainted with same.	
		11c. If examination is desired, place the slide, culture side up, on the microscope stage of a microscope equipped for oil immersion examination.	
		11d. Place a drop of a suitable bacteriological immersion oil on the area to be viewed (culture smear).	V.D.3.11.11d (p. 51)
		11e. The proper objective is positioned for oil immersion (usually labeled "oil" and having x 97 or x 100 magnification).  X20 ACHRO X100/1·3 OIL / 160 TYPICAL OIL OBJECTIVE OIL IMMERSION OBJECTIVE THE OBJECTIVES SCREW INTO THE TURRET. (TURN AND LOCK FOR SELECTION)	

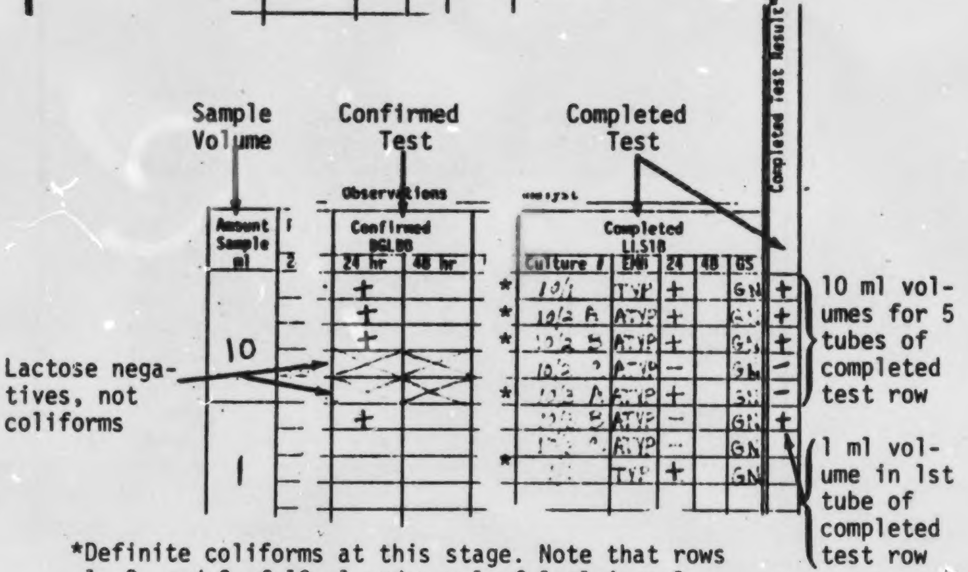
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedure (Continued)		11f. Turn ON lighting system. Light will be directed to reflect off the plane side of a mirror through a condenser assembly and up through the stage. (Mirror assembly may be external or internal.)	
		11g. With the illumination system correctly set up, rack down (or the stage up on some models), until the oil-immersion lens just touches and disperses the oil.	V.D.3.11.11g (p. 51)
		11h. Rack down gently with the coarse control (lens and slide will move toward each other) while looking down the microscope (into the eyepiece) until the image begins to come into focus.	
		11i. Obtain final sharp image using the fine-focus control.	
	EXAMINATION AND RECORDING OF STAINS		
	12. Examine stained preparation for bacteria.	12a. Gram-negative bacteria (typical of coliforms) will be red or pink colorations.	
		12b. Gram-positive bacteria (NOT coliforms) will be blue-to-purple in color.	
		12c. Mixed cultures will show mixtures of the above and will immediately call for the re-isolation of pure culture on another EMB agar plate from the saved nutrient agar slant. Discard the LLSTB tube as it has no interpretative value being a mixed culture. Repeat procedures as before.	
		12d. If two large of a sample was transferred to the slide for staining, some areas of matted, numerous bacterial cells could produce areas where dyes could not either penetrate or be washed away. Recommend another smear to be made.	
		12e. Examine each of the stains prepared.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedure (Continued)	13. Record gram stain data.	13a. Assume, for our examples, that only Gram negative (GN) organisms were observed during microscopic examination. 13b. Enter observations in proper place on data sheet:	



Completed LLSTB

8 hr	Culture #	EMB	24	48	GS
	10/1	TYP	+		GN
	10/2 A	ATYP	+		GN
	10/2 B	ATYP	+		GN
	10/2 C	ATYP	−		GN
	10/3 A	ATYP	+		GN
	10/3 B	ATYP	−		GN
	10/3 C	ATYP	−		GN
	1/1	TYP	+		GN



Sample Volume
Confirmed Test
Completed Test
Lactose negatives, not coliforms
10 ml volumes for 5 tubes of completed test row
1 ml volume in 1st tube of completed test row
Completed Test Result

Observations

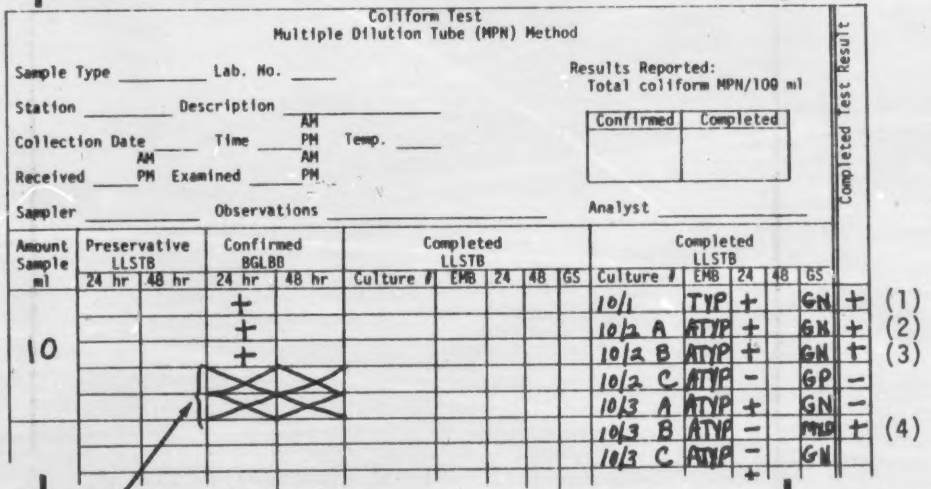
Amount Sample ml	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr
10	+	
	+	
	+	
	X	X
	X	X
1	+	

Analyst

	Completed LLSTB Culture #	EMB	24	48	GS	Completed Test Result
*	10/1	TYP	+		GN	+
*	10/2 A	ATYP	+		GN	+
*	10/2 B	ATYP	+		GN	+
	10/2 C	ATYP	−		GN	−
*	10/3 A	ATYP	+		GN	−
	10/3 B	ATYP	−		GN	+
	10/3 C	ATYP	−		GN	
*	1/1	TYP	+		GN	

*Definite coliforms at this stage. Note that rows 1, 2, and 3 of 10 ml and row 1 of 1 ml inoculation volume all have at least 1 coliform represented by culturing.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedure (Continued)		13c. Other entries could have been MXD (mixed culture) or GP (Gram Positive). 13d. In the case of a GP entry, the culture is not a coliform and no further action need be taken for the specific culture.	



Coliform Test
Multiple Dilution Tube (MPN) Method

Sample Type ________ Lab. No. ____
Station ________ Description ________________
Collection Date ____ Time ____ AM PM Temp. ____
Received ____ AM PM Examined ____ AM PM
Sampler __________ Observations ________________
Results Reported:
Total coliform MPN/100 ml

Confirmed	Completed

Analyst ____________

Amount Sample ml	Preservative LLSTB		Confirmed BGLBB		Completed LLSTB					Completed LLSTB					Completed Test Result	
	24 hr	48 hr	24 hr	48 hr	Culture #	EMB	24	48	GS	Culture #	EMB	24	48	GS		
10			+							10/1	TYP	+		GN	+	(1)
			+							10/2 A	ATYP	+		GN	+	(2)
			+							10/2 B	ATYP	+		GN	+	(3)
										10/2 C	ATYP	−		GP	−	
										10/3 A	ATYP	+		GN	−	
										10/3 B	ATYP	−		MXD	+	(4)
										10/3 C	ATYP	− +		GN		

Lactose negatives
Not coliforms

(1) 10 ml/first row Positive Confirmed Test...
GN lactose fermenter (culture 10/1)

(2) 10 ml/second row Positive Confirmed Test...
GN lactose fermenter (cultures 10/2 A and 10/2 B)

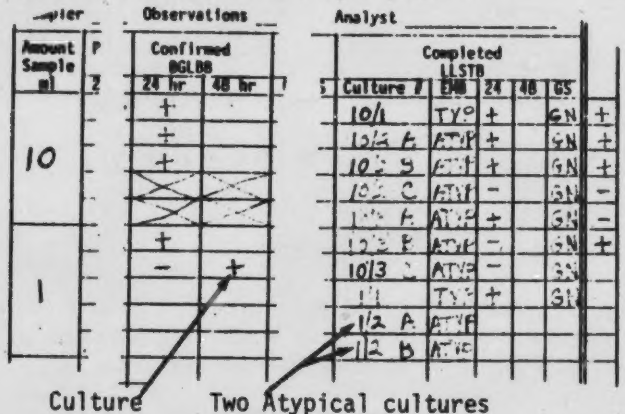
(3) 10 ml/third row Positive Confirmed Test...
GN lactose fermenter (culture 10/3 A)

Note: Culture 10/3 B and 10/3 C need no longer be processed since a positive is no longer needed from these respective cultures. Had

(Continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Third-Day Procedure (Continued)		culture 10/3 A been a negative for coliforms, however, the other two cultures must be processed to determine coliform content: 10/3 B Mixed culture EMB → $\begin{cases}\text{NAS}\\ \text{LLSTB}\end{cases}$ etc. 10/3 C Negative LLSTB...hold another 24 hours for possible fermentation (+) (4) 1 ml/first row Positive Confirmed Test... GN lactose fermenter (culture 1/1)	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Fourth-Day Procedure 1. Equipment Maintenance	1. Check, record, and adjust incubator.		
2. Lab Bench Disinfection	1. Disinfect laboratory bench.	1a. Sponge and disinfectant; paper toweling.	
3. Data Sheet Recordings	1. Locate required data sheet.	1a. Sample "217" is our example.	
	2. Remove cultures from incubator and assemble with data sheet.	2a. 1 EMB agar plate (#217 1/2), 24 hours old. 2b. Record necessary data regarding colony characteristics on EMB agar plate:	See "Data Sheet" D.3.3.3a



ampler Observations Analyst

Amount Sample ml	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr
10	+	
	+	
	+	
	X	X
	X	X
1	+	
	−	+

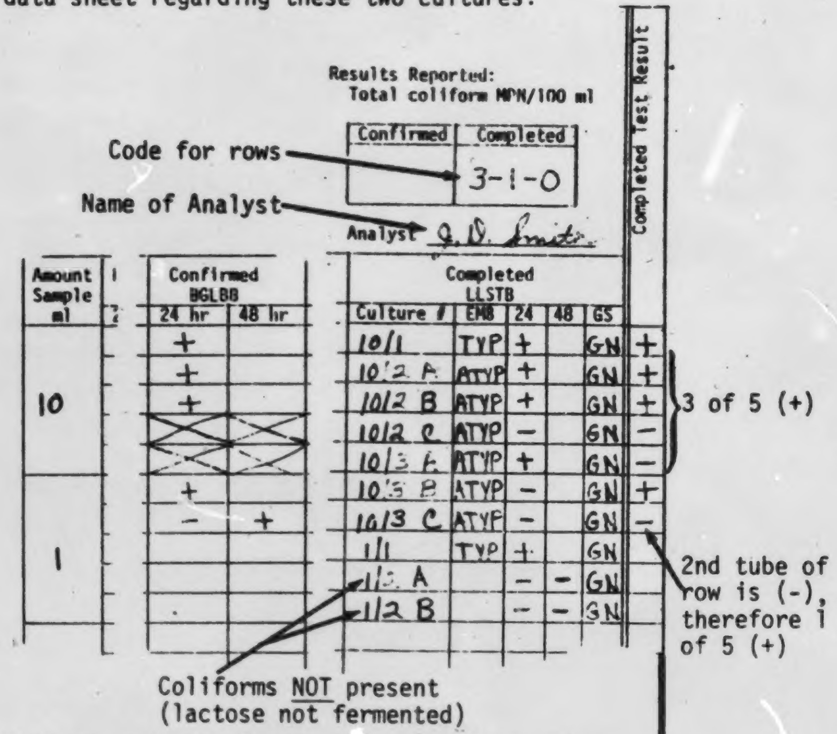
Culture #	EMB	Completed LLSTB 24	48	GS	
10/1	TYP	+		GN	+
10/2 A	ATYP	+		GN	+
10/2 B	ATYP	+		GN	+
10/2 C	ATYP	−		GN	−
10/3 A	ATYP	+		GN	−
10/3 B	ATYP	−		GN	+
10/3 C	ATYP	−		GN	
1/1	TYP	+		GN	
1/2 A	ATYP				
1/2 B	ATYP				

Culture 217 1/2

Two Atypical cultures to be processed to NAS and LLSTB

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. EMB Agar Plate Processing (Culture 217 1/2)	1. Transfer two of the atypical cultures to NAS and LLSTB.	1a. As per data sheet (1/2 A and 1/2 B). 1b. Use procedures outlined in C.5.1. 1c. Incubate cultures at 35°C ± 0.5°C.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Fifth/Sixth Day Procedures 1. Completed Test Termination	1. Completed test terminated with processing of 1/2 A and 1/2 B.	1a. Gram stains made in accordance with previous instructions. Recordings made. 1b. LLSTB observations and recordings made. 1c. Assume the following information added to the data sheet regarding these two cultures:	



Results Reported:
Total coliform MPN/100 ml

Confirmed	Completed
	3-1-0

Code for rows

Name of Analyst

Analyst J. D. Smith

Amount Sample ml	Confirmed BGLBB 24 hr	Confirmed BGLBB 48 hr	Completed LLSTB Culture #	EMB	24	48	GS	Completed Test Result
10	+		10/1	TYP	+		GN	+
	+		10/2 A	ATYP	+		GN	+
	+		10/2 B	ATYP	+		GN	+
			10/2 C	ATYP	–		GN	–
			10/3 A	ATYP	+		GN	–
1	+		10/3 B	ATYP	–		GN	+
	–	+	10/3 C	ATYP	–		GN	–
			1/1	TYP	+		GN	
			1/2 A		–	–	GN	
			1/2 B		–	–	GN	

3 of 5 (+)

2nd tube of row is (-), therefore 1 of 5 (+)

Coliforms NOT present (lactose not fermented)

1d. Had one or both cultures checked out as coliforms, the 2nd tube of the 2nd row would have been (+) and, therefore, resulted in 2 of 5 tubes positive (instead of 1 of 5 for this row), giving a 3-2-0 code.

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

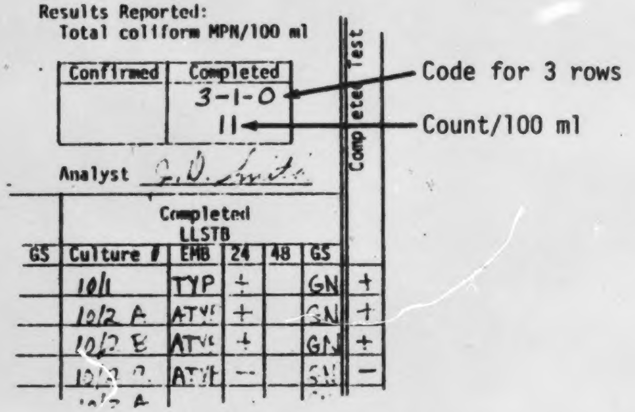
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Fifth/Sixth Day Procedures (Continued) 2. Interpretation of Test Results	1. Determine number of positives for the completed test.	1a. Results of confirmed test are not used since further and more conclusive testing has been done (completed test). 1b. Our example (F.1.1.1c) shows:	II.F.2.1 (p. 46)

Sample Volume	No. Positives
10	3
1	1
1/10	0

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	2. Look up and note the MPN index from the MPN table.	2a. For the given example (3-1-0) a typical table of MPN's will show an index of 11 as noted by arrow below:	Std. Meth. 14:923

MPN INDEX FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 10-ML PORTIONS, FIVE 1-ML PORTIONS AND FIVE 0.1 ML PORTIONS ARE USED

No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml
5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each	
0	0	0	<2
0	0	1	2
0	1	0	2
0	2	0	4
3	0	0	8
3	0	1	11
3	1	0	11 ←
3	1	1	14

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>F. Fifth/Sixth Day Procedures (Continued)</td><td>3. Record the calculated total coliform/100 ml completed test count on the data sheet.</td><td>3a. Value is direct index if, as our example, 10 ml portions were used in the first row. Other volumes used would have necessitated adjusting the index value.
3b. Record as follows:

Results Reported:
Total coliform MPN/100 ml
<table>
<tr><th>Confirmed</th><th>Completed</th></tr>
<tr><td></td><td>3-1-0
11</td></tr>
</table>
Code for 3 rows
Count/100 ml

Analyst G. D. Smith
<table>
<tr><th></th><th colspan="5">Completed LLSTB</th><th>Completed Test</th></tr>
<tr><th>GS</th><th>Culture #</th><th>EMB</th><th>24</th><th>48</th><th>GS</th><th></th></tr>
<tr><td></td><td>10/1</td><td>TYP</td><td>+</td><td></td><td>GN</td><td>+</td></tr>
<tr><td></td><td>10/2 A</td><td>ATY</td><td>+</td><td></td><td>GN</td><td>+</td></tr>
<tr><td></td><td>10/2 B</td><td>ATY</td><td>+</td><td></td><td>GN</td><td>+</td></tr>
<tr><td></td><td>10/2 C</td><td>ATYP</td><td>−</td><td></td><td>GN</td><td>−</td></tr>
<tr><td></td><td>10/3 A</td><td></td><td></td><td></td><td></td><td></td></tr>
</table></td><td>II.F.2.3.3a</td></tr>
<tr><td>G. Reporting of Results</td><td>1. Report results as prescribed under regulatory requirements.</td><td></td><td></td></tr>
</table>

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II*	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

*Training Guide materials are presented here under the headings marked *. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

F.2.1

For purely qualitative aspects of testing for indicator organisms, it is convenient to consider the tests applied to one sample portion, inoculated into a tube of culture medium, and the follow-up examinations and tests on results of the original inoculation. Results of testing procedures are definite: positive (presence of the organism/group demonstrated) or negative (presence of the organism/group not demonstrated). The combination of results is used in an application of probability mathematics to secure a single MPN value for the sample. The MPN value for a given sample is obtained through the use of MPN tables. Standard practice in environmental water testing is to plant 3 rows of tubes (15 tubes - 3 rows of 5 tubes each) with each row containing equal increments of sample/tube and usually having a tenfold sample dilution factor between rows.

F.2.3.3a

When the series of decimal dilutions is other than 10, 1.0 and 0.1 ml, use the following formula:

$$\text{MPN index (from table)} \times \frac{10}{\text{Largest quantity tested}}$$

$$= \text{MPN/100 ml}$$

Example: From a sample of water, 5 out of five 0.01-ml portions, 2 out of five 0.001-ml portions, and 0 out of five 0.0001-ml portions, gave positive reactions.

From the code 5-2-0 in the MPN table, the MPN index is 49.

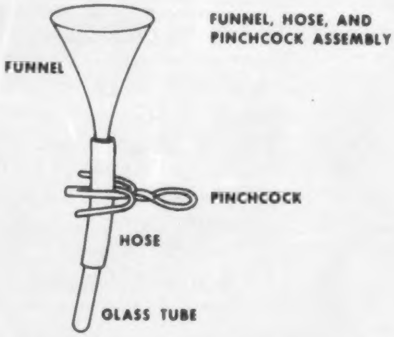
$$\underset{\text{(from table)}}{49} \times \frac{10}{0.01} = 49{,}000$$

$$\text{MPN/100 ml} = 49{,}000$$

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.4.1	A colony is defined as a discrete growth occurring at least 0.5 cm (approximately .2 inch) from any other growths. Such growths represent a large number of developmental successions from an original viable cell and therefore can be considered a "pure" culture. All organisms from pure cultures will exhibit the same characteristics when subjected to standard bacteriological testing.	
D.3.5	A gram staining procedure, in general, separates bacteria into two categories, gram positive (blue coloration) or gram negative (red coloration). Its usefulness to the coliform testing procedure is due to the fact that part of the coliform definition indicates that "gram negative, non-spore forming rods" are necessary, and, in addition, no gram positive organism must be present since some of these organisms can act "synergistically" (in conjunction with other non-coliforms) to produce a false positive result (gas production in lactose) which neither could manage independently. It is desirable to use known pure cultures of both a gram positive (staphylococcus, bacillus, etc.) and a gram negative (proteus, enterobacter, etc.) as controls for the staining procedure. A 24 hour culture is recommended for stained preparations since older cultures can give erroneous results.	

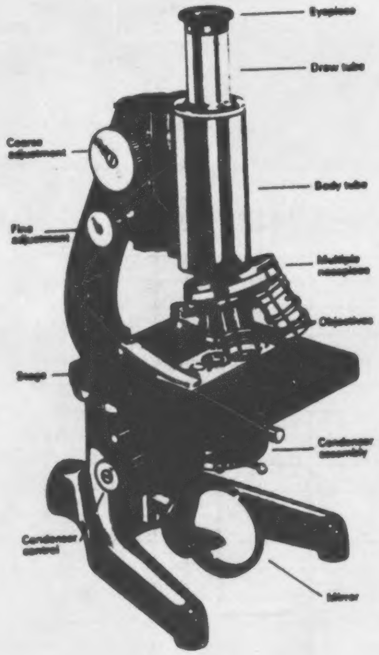
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1	Incubator must be of sufficient size for daily work load without causing crowding of tubes to be incubated. Considerations for choice of incubator type must relate to reliability of operation and not to cost or attractiveness of equipment.	
A.1.1	Incubator should be kept out of drafts or direct sunlight in order to prevent temperature inside the incubator from changing outside the temperature range specified (35° ± 0.5°). Power supply should be selected so that there will not be too many pieces of equipment on the same circuit. Otherwise, circuits will be blown repeatedly.	Standard Methods for the Examination of Water and Wastewater, 14th ed. (1975 APHA, WPCF, AWWA, p. 880 (Hereafter referred to as: Std. Meth. 14: (page no.)
A.1.2	Mercury bulb thermometer usually used in most incubators. Recording thermometer is acceptable, but, it should be calibrated against a mercury bulb thermometer which has been certified by National Bureau of Standards. The NBS certified thermometer always should be used with its certificate and correction chart.	
A.1.3	Saturated relative humidity is required in order to make the incubation more efficient (heat is transferred to cultures faster than in a dry incubator). Furthermore, culture medium may evaporate too fast in a dry incubator.	
A.1.5	Allow enough time after each readjustment to permit the incubator to stabilize before making a new adjustment. At least one hour is suggested.	
A.1.6	Incubator temperature can be held to much closer adjustment if operated continuously. Temperature records should be kept in some form of permanent record. A temperature record book is suggested with daily recording of values. If a recording thermometer is used, the charts may be kept as permanent record; if so, be sure that the charts are properly labeled to identify the incubator and the period covered. Uniform temperature (35°C ± 0.5°) is to be maintained on shelves in use.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.2.1-5	Since electric sterilizer will be operated intermittently, care should be taken that it is on a circuit which will not be overloaded when it is turned on. A time and temperature record is maintained for each sterilization cycle. Temperature recordings can be retained for records.	Std. Meth. 14:881
A.3.1	Autoclaves differ greatly in design and in method of operation. Some are almost like home-style pressure cookers; others are almost fully automatic. This is a subject which requires separate instruction; and should be related to the exact make and model of equipment you will use in your own laboratory. Vertical autoclaves and household pressure cookers may be used in emergency service if equipped with pressure gages and thermometers with bulbs positioned 1 inch above the water level. However, they are not to be considered the equivalent of the general purpose steam sterilizer recommended for permanent laboratory facilities. Their small size is inadequate for large-volume work loads, and they can be difficult to regulate. The following requirements must be met regarding autoclaves or sterilizing units: a. Reaches sterilization temperature (121°C), maintains 121°C during sterilization cycle, and requires no more than 45 minutes for a complete cycle. b. Pressure and temperature gages on exhaust side and an operating safety valve. c. No air bubbles produced in fermentation vials during depressurization. d. Record maintained on time and temperature for each sterilization cycle.	Std. Meth. 14:881
A.4.1-2	Distilled water in bacteriological laboratory must not contain substances which will prevent any bacteria from growing in culture medium in which the distilled water is used or will be highly nutritive. There are procedures for testing quality of distilled water; but these should be undertaken only by professional bacteriologists or in laboratories where this is done regularly. Use only glass stills or block tin lined stills.	Std. Meth. 14:645-49 14:888-91

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.5.1	pH Meter: See cited reference.	Std. Meth. 14:882
A.6.1-4a	Glassware: See cited reference on pipets and graduated cylinders, media utensils, bottles.	Std. Meth. 14:882-85
A.6.1-4b	Glassware can be checked for bacteriostatic or inhibitory residues by a bacteriological test procedure which, like the distilled water suitability test, should be undertaken only by professional bacteriologists or in laboratories where this test is done on a regular basis.	Std. Meth. 14:885
A.7.3	 FUNNEL, HOSE, AND PINCHCOCK ASSEMBLY FUNNEL PINCHCOCK HOSE GLASS TUBE NOTE: UNIT NEED NOT BE STERILE FOR MEDIUM DELIVERY ONLY.	
A.8.2c	Some workers prefer to utilize a magnetic swirl bar and hot plate arrangement. This is acceptable and will require no agitation until the medium is near boiling at which time the swirling action should be terminated and the medium gently swirled by hand and the flask monitored for boiling.	
B.6.5	Alternately, it is authorized to use an "inoculation stick" for transfers and plate streaking. A precisely sized and sterilized stick is intended for a one-time use and, if used, eliminates the need for a burner during the transfer procedure. Of course, several will have to be used during the streaking process since a "sterile" one is required during the streaking carry-over to sterile surfaces. Also available are re-sterilizable loops used once, resterilized, and available for future transfers.	Std. Meth. 14:917 Std. Meth. 14:883-84

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

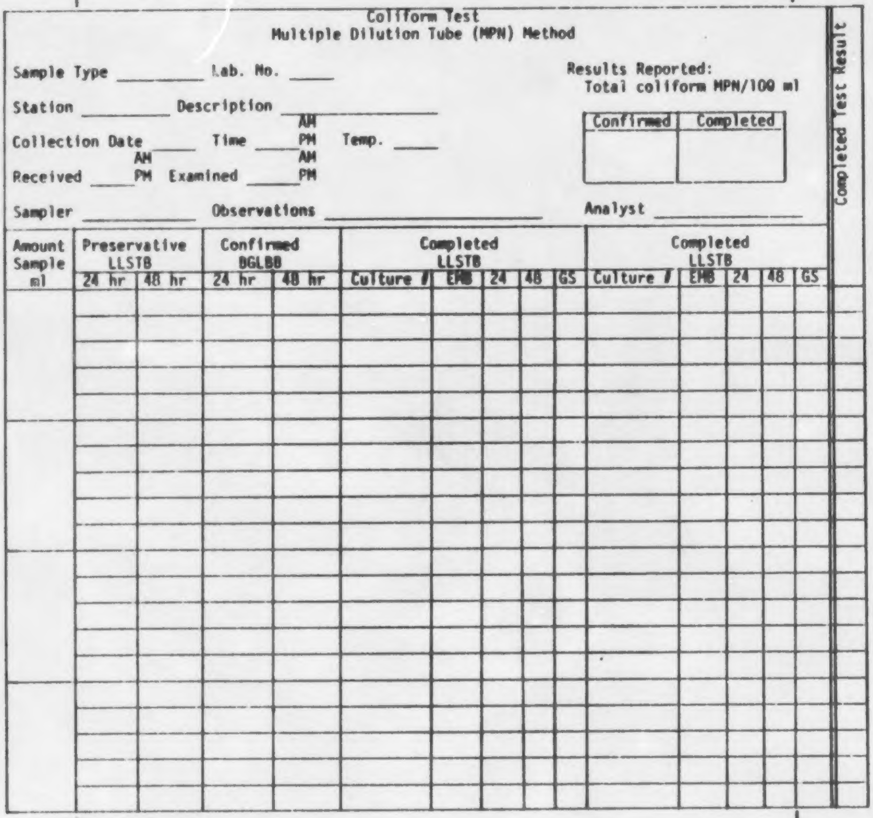
D.3.11



D.3.11.11d Some manufacterurs specify that the upper most lens of the condenser assembly also be coated with a drop of oil prior to placing the slide on the stage. In effect, this would be "sandwiching" the slide between two oil interfaces through which the light must pass.

D.3.11.11g It is extremely important to properly set up the illumination system for proper results. Procedures vary according to the type of illumination provided, the type of diaphragm used, and the controls provided by the particular microscope. Final results would give, if accomplished correctly: correct lighting from the light source; centrally placed optimal lighting; and a sharply focused image.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.2	There is no such thing as a "standard" data sheet for bacteriological tests. A suggested data sheet is shown below:	



Coliform Test
Multiple Dilution Tube (MPN) Method

Sample Type ________ Lab. No. ____

Station ________ Description ______________

Collection Date _____ Time _____ AM/PM Temp. ____

Received _____ AM/PM Examined _____ AM/PM

Sampler __________ Observations ____________________

Results Reported:
Total coliform MPN/100 ml

Confirmed	Completed

Analyst ______________

Amount Sample ml	Preservative LLSTB		Confirmed BGLBB		Completed LLSTB					Completed LLSTB					Completed Test Result
	24 hr	48 hr	24 hr	48 hr	Culture #	EMB	24	48	GS	Culture #	EMB	24	48	GS	

	Note: This data sheet could also be used exclusively for the confirmed test and not for only the completed test stage.	
B.6.6	There is no standardized way to accomplish a streak plate in order to isolate pure cultures. Some workers prefer to carry the streaks around the plate several more times with its attendant loop sterilizing sequencing between each of the streakings. Others prefer to use a specially made petri dish which features a center partition which "halves" the dish allowing two separate cultures to be cultivated. These modifications, and others, are not deviations since the only consideration which matters is that a pure culture is available for further testing.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
D.3.5	You will note that three cultures (10/2 C; 10/3 B; and 10/3 C) are being processed for gram staining even though their 24 hour LLSTB results are negative. This is done for the following reasons: 1. Growth used after 24 hours may give erroneous staining patterns. 2. Staining is quickly accomplished and is preferable over restreaking a NAS and waiting an additional day for culturing. 3. Microscopic examination need not be done after staining and can wait for the 48 hour fermentation tube results...if positive, proceed microscopically; if negative, coliforms absent and discard stained slide.	

This outline was prepared by: Rocco Russomanno, Microbiologist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

A PROTOTYPE FOR THE DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

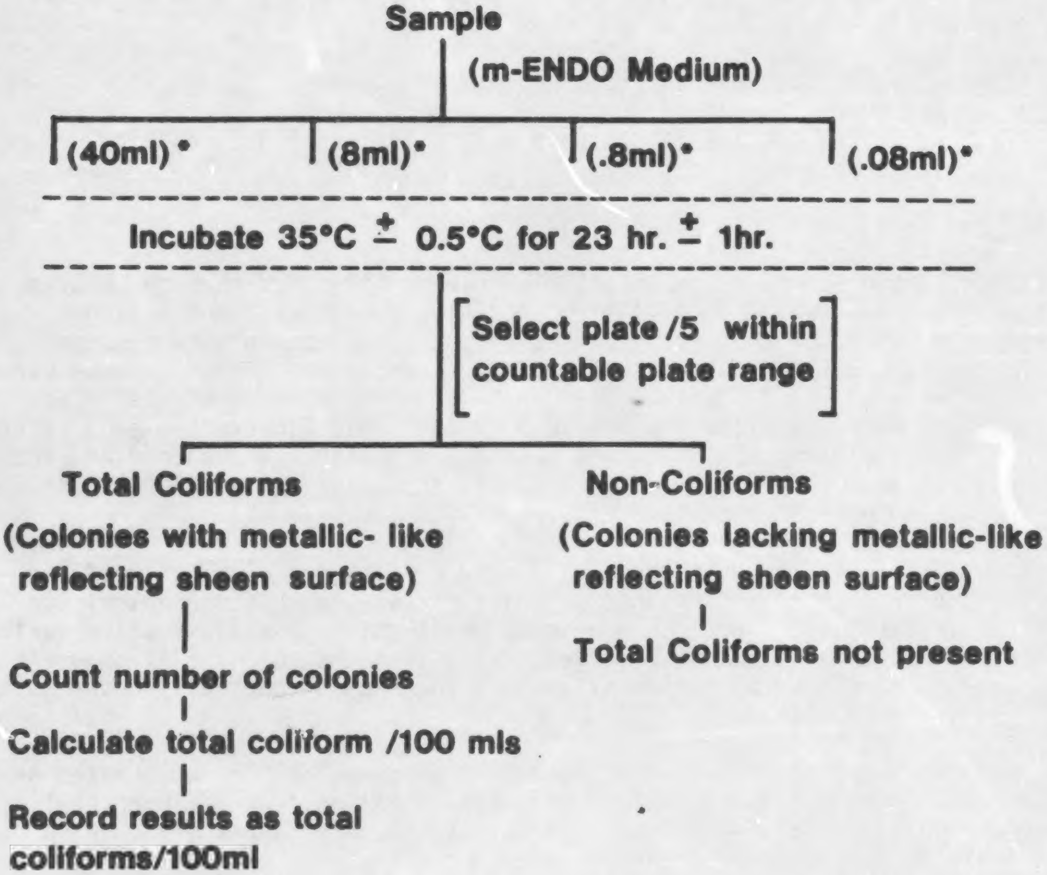
TOTAL COLIFORM TEST BY THE MEMBRANE FILTER METHOD

as applied in

WATER TREATMENT FACILITIES
WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

BA.MET.lab.WMP.9.5.78


Sample
(m-ENDO Medium)
(40ml)*
(8ml)*
(.8ml)*
(.08ml)*
Incubate 35°C ± 0.5°C for 23 hr. ± 1hr.
Select plate /5 within countable plate range
Total Coliforms
(Colonies with metallic- like reflecting sheen surface)
Count number of colonies
Calculate total coliform /100 mls
Record results as total coliforms/100ml
Non-Coliforms
(Colonies lacking metallic-like reflecting sheen surface)
Total Coliforms not present

*Note: Since sample volumes necessarily change dependent upon the existing water quality, these have been arbitrarily selected to give a cross-section of laboratory procedural methodology

WATER MONITORING PROCEDURE: Total Coliform Test by the Membrane Filter Method

1. Analysis Objectives

In environmental water monitoring, the application of this methodology can be for any of the following:

a. Test parameter for the conduction of a sanitary survey during an on-site review of the water source.

b. Monitoring of influent waters of treatment plant.

c. Test parameter for special purpose waters (recreational, boating, controlled loading, etc.).

2. Brief Description of Analysis:

Multiple portions of a representative sample are filtered through membrane filters contained within a filtering apparatus. Bacteria in the sample portions are held on the upper surface (gridded) of each of the membranes, while the sample water passes through and is discarded. After several rinses of the funnel of the filtering apparatus with sterile buffered distilled water, each membrane filter is placed on a paper pad (absorption pad) saturated with a medium called m-ENDO Broth within a petri dish. The closed end inverted dishes are incubated within a high humidity incubator set at 35°C ± 0.5°C for an incubation period of 22-24 hours.

On this medium, coliform bacteria will grow and develop a golden metallic sheen-like surface on the colonies. Colonies lacking this characteristic reflective surface are not considered as coliforms. This distinctive surface sheen may appear at the center, edges or all-over the colony. At times it can form as flecks or particles of sheen throughout or partially covering the colony.

The membranes are inspected with the aid of a microscope or lens having a magnification of 10x to 15x under reflective lighting from a fluorescent source. Coliform colonies, if any, from suitable membrane/s are counted and a calculation made to determine total coliforms per 100 millimeters.

3. Applicability of this Procedure:

a. The range of total coliform concentrations:

If the sample volumes used are	These ranges of total coliforms covered are
40 ml to .08 ml	50 to 100,000/100 ml

b. Pretreatment of samples in accordance with Standard Methods, 14th Ed. (p. 904-907)

This procedure conforms to the Standard Total Coliform MPN Tests as described in Standard Methods for the Examination of Water and Wastewater, 14th ed. (1975). p. 914

WATER MONITORING PROCEDURE: Total Coliform Test by the Membrane Filter Method

Equipment and Supply Requirements

A. Capital Equipment:

1. Autoclave, steam, providing uniform temperatures up to and including 121°C and equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperatures within 30 minutes. (Alternately, a suitable pressure cooker is acceptable--see Standard Methods for particulars.)
2. Incubator, air, providing uniform and constant temperature of 35°C. ±0.5°C and having an atmosphere of at least 90% relative humidity.
3. Oven, hot-air, providing uniform temperatures within the range of 160-180°C
4. Apparatus, water distillation, distilled water product suitable for bacteriological operations (alternately, a suitable source is permissible).
5. Microscope, stereoscopic, 10X to 15X magnification with fluorescent lighting mandatory. (Alternately, a suitable magnifying lens with fluorescent lamp is acceptable.)
6. Refrigerator, set for less than 10°C but above the freezing temperature.
7. Vacuum source, preferably a pump assembly with suitable hoses and shut-off valve provided. (Alternately, an aspirator or hand pump with the same provisions are acceptable.)
8. Balance, analytical, sensitivity of 1 mg.
9. Gas source, suitable for burner. (Alternately, an alcohol lamp can be used.)

B. Reusable Supplies:

1. Apron, suitable for laboratory operations.
2. Bottle, sample, of sufficient size for standard sample, preferably of 250 ml, wide-mouth, glass stopper, with tag. (Alternately, 120 ml size)
3. Bottle, squeeze type, containing disinfecting solution.
4. Burner, gas, suitable for laboratory operations with connecting hose.
5. Thermometer, NBS (or NBS calibrated), functions within 20°-60°C range with individual markings of 1°C.
6. Thermometer, NBS (or NBS calibrated), functions within 150°-190°C range with individual markings of 1°C.
7. Filtration Unit, MF, a seamless funnel attached to a receptacle bearing a porous plate (screen, porous disc, etc.) and constructed from stainless steel, glass, porcelain, plastic, or other suitable material.
8. Hot plate, controllable heat range up to the 100°C range.
9. Balance, trip, sensitivity of 0.1 gram at a load of 150 grams, with appropriate weights.
10. Meter, pH, accurate to within 0.1 pH unit, with suitable standard pH reference solution(s).
11. Can, pipet, non-toxic and sterilizable material (if pre-sterilized disposable type pipets are used, this item is unnecessary).
12. Pan, discard, receives contaminated material and pipets and contains disinfectant. Should be of sufficient length to receive pipets placed horizontally.
13. Cylinder, graduated, 500 ml, 100 ml, 50 ml, and 25 ml size. (The 50 ml size is covered with a "cap" of foil or Kraft paper and then sterilized.)

Equipment and Supply Requirements (Continued)

14. Blank, dilution water, 99 ml.
15. Pipets, microbiological, 50. ml, with 0.1 ml graduations, sterile cotton plugged, glass or disposable types (the disposable types are for one time use and may be glass or plastic).
16. Pipets, microbiological, 1.0 ml, with 0.1 graduations, sterile cotton plugged, glass or disposable types (the disposable types are for one time use and may be glass or plastic).
17. Pipets, microbiological, 10 ml, with 1 ml graduations, sterile, cotton plugged, glass or disposable types (the disposable types are for one-time use and may be glass or plastic).
18. Beaker, 50 ml (for measuring pH).
19. Flask, volumetric, 1 liter capacity (for stock solution of phosphate buffer).
20. Flask, Erlenmeyer, 500 ml capacity (for holding buffered distilled rinse water).
21. Flask, sidearm, 1 liter size (for reservoir of MF apparatus; proper size bored, rubber stopper is needed to connect MF filtration flask to flask and hose required to vacuum source (must be rigid enough to avoid collapse under vacuum and flexible enough to be controlled by pinch clamp) pinch clamp - vacuum control.
22. Flask, Erlenmeyer, 50 ml (for preparing m-ENDO medium).
23. Forceps, curved end, round tip.
24. Bottle, small, Methanol or Ethanol volume to cover ends of forceps.
25. Sponge, small, to spread and wipe germicide.
26. Desiccator, media storage, ideally opaque or darkened and containing desiccating agent to remove moisture.

C. Consumable Supplies:

1. Dish, petri, disposable, tight fitting plastic, 50 x 12 mm, sterile.
2. m-ENDO Broth, medium, dehydrated, total coliform. Distributors, Difco, BBL, or other equivalent preparation.
3. Pencil, wax, recommended of soft wax equivalent to Blaisdell 169T.
4. Tags, bottle marking.
5. Glass Wool.
6. Cotton, non-absorbent.
7. Paper, Kraft wrapping.
8. Foil, aluminum, heavy duty.
9. Matches or striker.
10. Towels, paper.
11. Detergent, non-toxic, laboratory cleaning
12. Data Sheet, as required by analyst's agency.
13. Filter, membrane, 47mm, 0.45 µm pore size, white, grid marked, sterile.
14. Pad, absorbent, 48 mm, sterile (usually included with membrane packet).
15. Potassium Dihydrogen Phosphate (KH_2PO_4), recommended 1/4 lb.
16. Sodium Thiosulphate ($Na_2S_2O_3SH_2O$).
17. Disinfectant, for bench tops and decontaminating purposes, bleach of household strength and prepared according to label directions.
18. Sodium Hydroxide (NaOH), 1N.
19. Distilled water, suitable for bacteriological operations. Obtainable from distillation apparatus (see Capital Equipment) or suitable source of supply.

Equipment and Supply Requirements (Continued)

20. Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$).
21. Ethanol, 95%.

Item needs in quantities or required size or space allowances cannot be specified, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of Standard Methods for the Examination of Water and Wastewater.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures		Aa. All pretest procedures completed before starting other first-day procedures.	
1. Incubator Setup, Adjustment (35°C ± 0.5°C)	1. Place incubator in permanent location.	1a. Floor location for large unit or table or bench for smaller units. 1b. Out of drafts or place in which it will be in direct sunlight part of day. 1c. Location convenient to laboratory operations. 1d. Convenient source of electric power with a separate circuit, if possible.	V.A.1.1
	2. Provide a saturated humidity within incubator.	2a. Check manufacturer's handbook for maintenance of humidification system, if installed. 2b. If humidifier system not installed within incubator, place beakers or trays containing distilled water on shelves to provide relative humidity of at least 90 percent during operating temperatures.	III.A.1.2b
	3. Install thermometer.	3a. Functions at least in 30°-40°C range. Meets NBS standards. Have 0.2°C increment markings or less. 3b. Usually a corner location to prevent breakage and tip immersed in a bottle containing water, glycerin, etc. for a more stable reading. 3c. If thermometer assembly has been installed by manufacturer, check for above requirements and calibrate with NBS thermometer. Calibration may be possible by removal and testing of installed unit or by comparison during incubator operation.	V.A.1.3
	4. Connect incubator to electric power source and turn ON.	4a. Pilot light should come on.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for location and method of temperature adjustment. 5b. Allow about 1 hour between fine adjustment (less than 2 degrees) and immediate adjustments can be made when the calibration is greater than this amount. Temperature achievement by the setting knob will be usually indicated by either a light indication or by an alternate lighting of a "heat-ON" - "refrigerant-ON" or other arrangement depending upon the incubator type/model.	
	6. Operate incubator continuously.	6a. Operate incubator continuously unless it will be unused for a relatively long period. (2 weeks or more). 6b. Daily check of temperature required, preferably an early morning and late afternoon with a written record maintained. Adjust temperature if necessary. 6c. Check at least biweekly the humidity level of interior of incubator. Add water to humidifier unit, if applicable, or to trays placed on the shelves providing humidification by convection.	
2. Oven Sterilizer-Drier Setup, Adjustment (170° ± 1.0°C)	1. Place oven sterilizer-drier in permanent location.	1a. Convenient source of electric power.	V.A.2 (p. 38)
	2. Connect oven/drier to power source and turn ON.	2a. Usually an indication is given that power is applied--such as an indicator light.	
	3. Install thermometer.	3a. If installed by manufacturer, ascertain if installation meets the above requirements.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	4. Adjust oven temperature to stabilize at required sterilizing temperature.	4a. 170°C is the required temperature. Arbitrarily, for this publication, a 1 degree leeway is stipulated. 4b. Manufacturer's instruction for location and method of temperature adjustment. 4c. Allow about 1 hour between fine adjustments (less than 2 degrees of desired temperature) and immediate adjustments can be made when the calibration is greater than this amount.	
3. Autoclave Setup	1. Install and operate autoclave according to manufacturer's instructions.	1a. Variable in design and operation, and unless properly operated can be dangerous. 1b. Used to sterilize objects made of or including liquids, rubber, and some plastics, and, for glassware, if desired. 1c. Operated for general sterilization at 121°C. (250°F) for a period of 15 minutes after this temperature has been attained. 1d. Sterilized media and liquids must be removed as soon as possible upon completion of sterilization from the chamber of the autoclave.	
4. Water Distillation Equipment Setup	1. Install and operate in accordance with manufacturer's instructions.	1a. Must produce water meeting quality requirements for bacteriological tests.	V.A.4 (p. 39)
	2. Operate as required to maintain adequate supplies of suitable distilled water.		
5. pH Meter Setup	1. Setup and operate in accordance with manufacturer's instructions.	1a. Meter must be accurate to at least 0.1 pH unit.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued) 6. Glassware Preparation	1. Clean and rinse using a suitable detergent and hot water.	1a. Nontoxic detergent must be completely removed from glassware.	
	2. Use final rinsings of deionized or distilled water.	2a. Six to twelve successive rinsings may be required. 2b. Must produce a clean dry glassware which meets bacteriological requirements for suitability.	
7. Sample Bottle Preparation	1. Deliver 0.1 ml or 0.2 ml of 10% sodium thiosulfate solution to each sample bottle (0.1 ml to 4 oz. or 120 ml size and 0.2 ml to 6-8 oz. or 250 ml size).	1a. Bottle meets glassware requirements. 1b. Use 1 ml pipet. 1c. Provides adequate sodium thiosulfate for neutralizing chlorine in sample. Note: If the sample does not contain chlorine, it is not necessary to add the sodium thiosulfate.	
	Sodium thiosulfate is prepared as follows:	Sodium Thiosulfate Preparation	
	*Weigh 10.0 grams of sodium thiosulfate.	1d. Use of trip balance for weighing acceptable.	
	*Dissolve in 50-60 ml of distilled water.	1e. 100 ml graduated cylinder satisfactory for volume measurements.	
	*Add distilled water to bring final volume to 100 ml.	1f. Final preparation should be labeled as 10% Sodium Thiosulfate and stored in refrigerator.	
	*Transfer to labeled bottle.		
	2. Place cover on sample bottle.		
	3. Place paper or metal foil cover over bottle cap or stopper.	3a. Protects opening of sample bottle from accidental or natural contamination.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	4. Sterilize sample bottle in autoclave or oven.	4a. In oven at 170°C for 1 hour. In autoclave at 15 pounds for 15 minutes.	
	5. Store sample bottle in clean dry place until used.		
8. Pipet Preparation	1. Insert a plug of non-absorbent cotton into mouthpiece of clean, dry pipet.	1a. Pipets which have chipped or broken tips or tops should be discarded. 1b. Cleanliness and suitability of pipets equivalent to bacteriological suitability of glassware. 1c. Cotton plug must be tight enough to prevent easy removal, either by pipeting action or by handling and yet be loose enough to permit easy air movement through the plug. 1d. Plug protects user from ingesting sample into his mouth.	
	2. Pass plugged end of pipet quickly through burner.	2a. Removes wisps of cotton which interferes with fingertip control of pipeting action.	
	3. Insert a layer of glass wool or multi-layer of paper padding in bottom of pipet can.	3a. Protects tips from damage. 3b. Pipets can be sterilized individually, if desired by wrapping in Kraft paper, then oven sterilizing. This technique would make the use of pipet cans unnecessary.	
	4. Place pipet in pipet can with delivery tip downward and contacting glass wool or paper. Close can when full or desirable to complete preparation.	4a. Cotton-plugged mouthpiece in pipeting is finger control end with the delivery tip on the opposite end. 4b. Approximately twenty (20) 1 ml pipets or twelve (12) 10 ml pipets will normally be accommodated in these cans. 4c. Can must be able to withstand sterilizing conditions. Toxic materials, such as copper, must not be used. Aluminum, stainless steel, or glass (Pyrex) are acceptable.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	5. Sterilize pipets.	5a. At least 1 hour in oven at 170°C, or 5b. In autoclave at 15 pounds steam pressure for 15 minutes. Cans removed quickly from autoclave after sterilizing with aid of asbestos gloves and opened quickly and slightly to allow residual steam to escape for a few seconds.	
	6. Store cans in a clean dry place until needed.		
9. Blanks, Dilution Water	1. Prepare stock solution of potassium dihydrogen phosphate (KH_2PO_4) by dissolving 34.0 grams of this chemical in 500 ml of distilled water and adjusting its pH to 7.2 with 1N NaOH. Dilute to 1 liter in volumetric flask.	1a. Distilled water may be measured in 500 ml graduated cylinder. 1b. Label to show contents, identity of preparer, and date of preparation. 1c. Stored in refrigerator. 1d. Discarded if mold or turbidity appear.	
	2. Prepare stock solution of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) by dissolving 50 grams of this chemical in 500-600 ml of distilled water and, after complete dissolving, bring the final volume to 1 liter in a volumetric flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	3. Prepare working solution of dilution water by adding 1.25 ml of the potassium dihydrogen phosphate stock solution and 5 ml of the magnesium sulfate stock solution to each liter of distilled water to be used in the preparation of dilution water.	3a. A 10 ml or 5 ml pipet is satisfactory for delivery of both of these stock solutions provided that it has graduation marks to deliver the proper amount. Use separate pipets for each solution to prevent contamination.	
	4. Deliver enough working solution to each dilution water bottle so that after sterilization the bottle will contain 99 ± 2 ml of dilution water.	4a. Recommended that dilution water bottles have a marking at the desired 99 ml quantity. Amount to be delivered to bottle before sterilization cannot be stated exactly as evaporation is different with differing conditions and autoclaves. Ordinarily about 102 ml will be required.	
	5. Place caps on bottles loosely.		
	6. Sterilize in autoclave.	6a. 15 minutes at 121°C.	
	7. Remove from autoclave, tighten bottle caps; cool to room temperature.		
	8. Store in cool place.	8a. Dilution bottles ready for use. May be stored indefinitely. 8b. Some evaporation loss may occur in time and in these cases, sterile similarly prepared water may be added. This is why a calibrated marked bottle is desirable.	

WATER MONITORING PROCEDURE: Total Coliform Test by the Membrane Filter Method

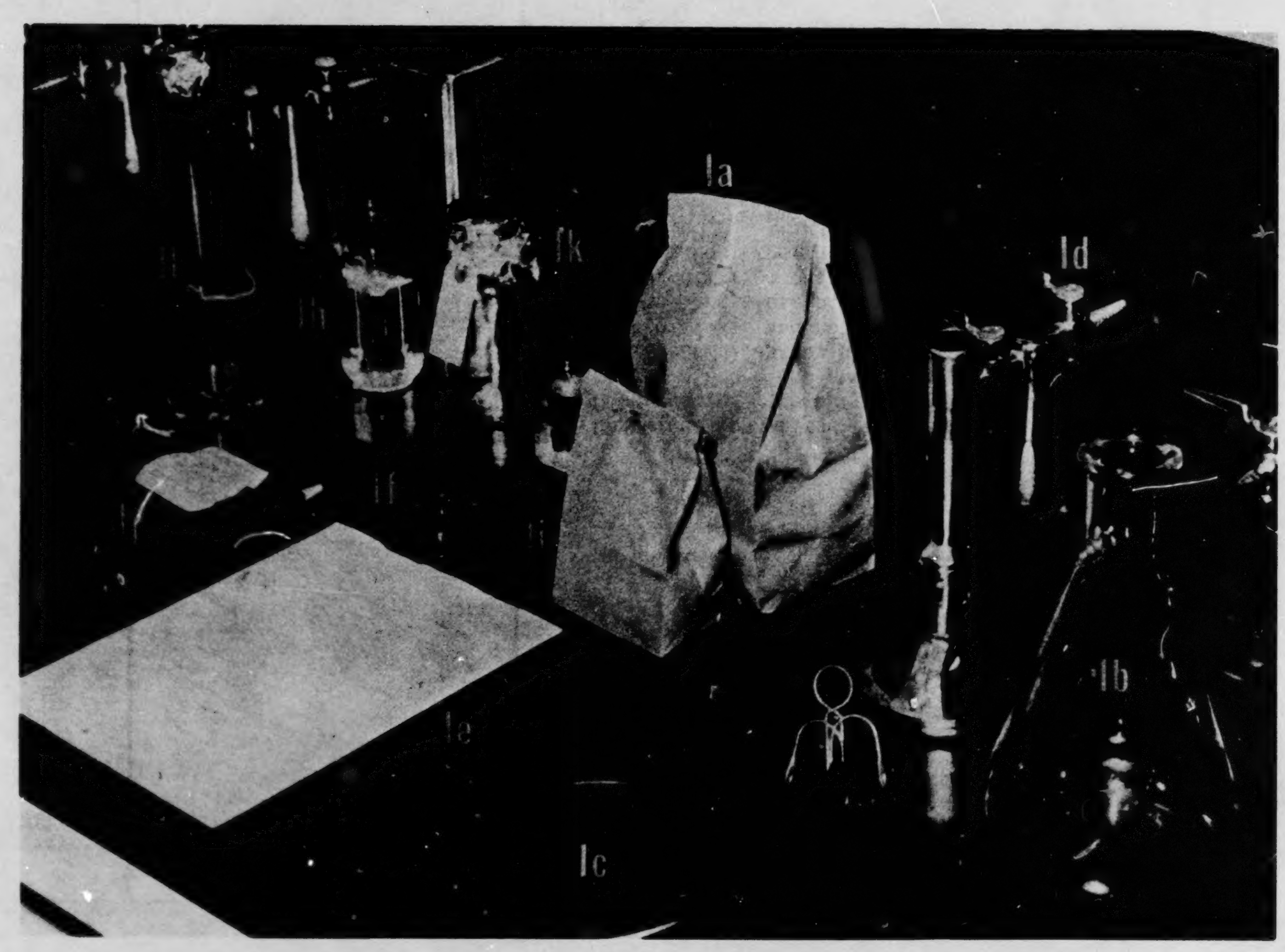
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued) 10. Preparation of Total Coliform Medium	1. Retrieve and inspect dehydrated m-ENDO broth medium.	1a. Best stored in desiccator which prevents moisture from damaging medium. 1b. Powder must be light pink without signs of hardening or color change to blood red hue.	VI.A.10.1 (p. 41)
	2. Weigh 1 gram of dehydrated m-ENDO broth.	2a. Sufficient for 20 ml of medium which prepares 10 petri dishes. 2b. Analytical balance having a sensitivity of 1 mg will be required. 2c. More than 2 grams being weighed can be done on less sensitive balance. This would provide more plates, but, of course, some medium can be discarded.	II.A.10.2 (p. 36)
	3. Place powder in a clean, dry 50 ml Erlenmeyer flask.		
	4. Prepare an alcohol-water solution as follows: a. Place 0.4 ml of ethanol in a clean, dry 25 ml graduate. b. Add distilled water to the graduate to the 20 ml mark.	4a. Graduate need not be sterile. No acceptable substitutes for ethanol. Use 1 ml pipet graduated in 0.1 ml increments. 4b. A Squeeze bottle addition to the graduate makes control of the distilled water addition easier.	VI.A.10.4 (p. 41)
	5. Add a small amount of the ethanol-water solution to the powder in the flask (about 5 ml). Swirl flask to mix powder and then add the remainder of the water.	5a. Small addition of water makes it easier to remove powder from walls of flask.	

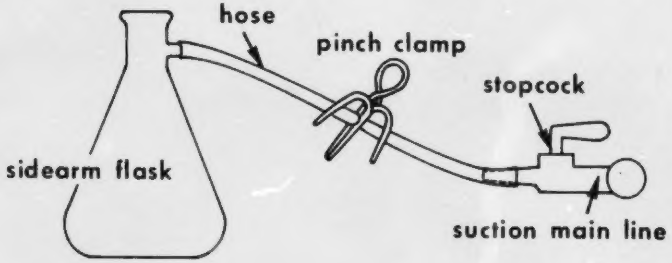
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	6. Cover top of flask with aluminum foil.	6a. Some laboratories use a screw-cap to cover the flask. If this is to be practiced, make sure that the cap is LOOSE when heating to relieve pressure built up during heating.	
	7. Heat flask on a hot plate set to high heat range.	7a. Constant stirring is necessary to prevent charring or burning of medium.	
	8. Remove at first sign of boiling.	8a. Prolonged heating reduces selectivity of medium. 8b. Do not autoclave this medium. 8c. Medium ready for use. Can be stored in refrigerator for up to 96 hours before discarding.	
11. Preparation of m-ENDO Plate	1. Remove a sterile petri dish from its container. Loosen its cover without removal.	1a. Usually in a sleeve of pre-sterilized plastic one-time-use dishes. 1b. Laboratory reusable sterilized glass dishes can alternately be used.	
	2. Remove a sterile absorption pad from its container and place in dish. Replace cover which is still kept loosely fitting.	2a. Usually packaged with membrane filters, or, alternately, can be separately laboratory sterilized. 2b. Use a forceps which has been standing in a flask of alcohol and then passed quickly through a flame to remove residual alcohol to handle the pad.	
	3. Transfer approximately 2 ml of the m-ENDO broth to the absorption pad within the dish.	3a. Plate can be stored in refrigerator for up to 96 hours before discarding or used immediately.	
	4. Gently tip the opened petri dish until a droplet of medium forms on the inner lower edge.	4a. A 2 ml broth addition is usually an excessive amount. 4b. Hold petri dish cover in other hand. Do not allow it to become contaminated.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures (Continued)	5. Gently shake out large droplet to waste. Replace cover tightly.	5a. Plate is ready for use in analysis. 5b. Keep plate from excessive exposure to light, particularly sunlight. 5c. It has been found that this procedure will invariably give an optimum amount of medium, whereas, trying to measure precisely the same amount for each plate will give deviations from optimum amounts more frequently. 5d. If plate is to be used within the hour simply cover with a paper towel on the bench. If a greater time is expected, place in refrigerator until used.	
B. First Day Procedure 1. Equipment Maintenance	1. Check, record, and adjust, if necessary, the 35°C incubator.		
	2. Check, record (if done) and adjust (if necessary) the refrigerator.		
2. Sample Collection and Handling	1. Collect sample, use a grab, direct filling, or suitable device collection technique.	1a. Representative of water supply system. 1b. Leave sufficient air space in bottle to allow shaking of sample (at least 2.5 cm or 1 inch). 1c. Do not rinse bottle before collecting sample as this would cause loss of dechlorinating agent. 1d. Exercise care to prevent contamination of samples.	
	2. Apply label to bottle and enter required information.	2a. Enter required information as per agency requirements. A minimum useful amount of entries include: *name of sampler (complete name, not initials) *location/code of collection site *time of collection *chlorine residual (water before sampling) *date of collection.	

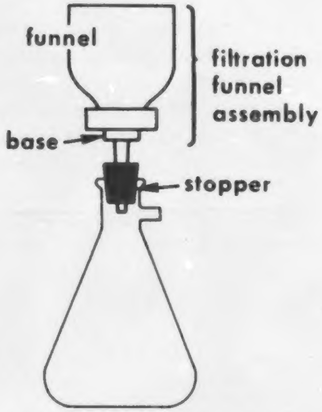
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)	3. Place bottle in closed ice chest.		
	4. Transport to laboratory and dispose of sample in accordance with laboratory policies.	4a. Sample should be analyzed as soon as possible. Immediate analysis is best but up to 6 hours holding time is acceptable.	
3. Preanalysis Preparation	1. Prepare laboratory data sheet.	1a. No standard data sheet. Use form recommended by laboratory/agency. 1b. Some of required information will be on sample label.	
	2. Disinfect laboratory bench; wipe dry.	2a. Use sponge and disinfectant; paper toweling.	
4. Equipment and Material Preparation/Assembly	1. Assemble required equipment and material.	1a. Filtration funnel assembly, sterile. 1b. Side arm suction flask, 1 liter size. 1c. Hose, suction w/clamp. 1d. Vacuum source, operational. 1e. Sheet, data. 1f. Prepared m-ENDO dishes. (4 required) 1g. Membrane filters, sterile. 1h. 99-ml buffered, distilled water blank. 1i. Forceps and disinfectant container (methanol). 1j. Pencil, marking. 1k. Sample bottle. 1l. Graduate, sterile, 50 ml, foil hood protected. 1m. Burner, gas, w/hose joined to gas source. * pipets, 10 ml, 1 ml sterile. (not shown) * buffered distilled rinse water (about 250 ml per analysis).	

WATER MONITORING PROCEDURE: Total Coliform Test for Drinking Water by the Membrane Filter Method

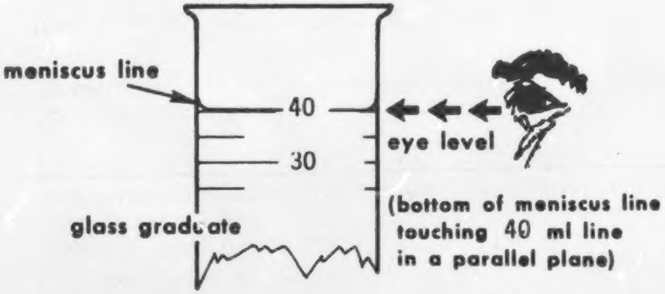


OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)	2. Assemble the units of the filtration apparatus.	2a. Suction side of apparatus may, depending on choice of equipment, consist of sidearm suction flask, suction line, pinch clamp, and suction device. Such an arrangement is shown below:  hose pinch clamp stopcock sidearm flask suction main line	V.B.4.2 (p. 39)
	3. Test the filtration apparatus for operation.	3a. Check suction units for cleanliness and operation: Open suction line by turning on stopcock and removing pinch clamp and check for suction at neck of sidearm flask by placing palm of hand over neck of flask and noting presence of suction. Replace and close pinch clamp and note if suction is cut off from the flask. NOTE: This test is made without the filtration funnel assembly being installed.	
	4. Assemble the units of the filtration unit assembly: Unwrap sterile funnel base from wrapping and place on base unit.	4a. The filtration unit assembly consists of a funnel and a base which should be clean, sterile, and in operational status. 4b. Manufacturers usually provide kits for maintenance of units. 4c. Do not contaminate working areas of funnel assembly (screen, inner area of funnel, funnel lip, etc.). 4d. Stopper may be retained on base of filtration unit throughout the usage and sterilization of the base.	

WATER MONITORING PROCEDURE: Total Coliform Test by the Membrane Filter Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)		4e. Units of filtration assembly are sterilized by steam after wrapping in Kraft paper or aluminum foil.  	
	5. Light burner.	5a. Some laboratories use an alcohol lamp.	
	6. Label m-ENDO plate with necessary identification markings.	6a. Conforms to data sheet. (See flow sheet.)	
5. Sample Filtrations A. 40 ml Volume	1. Place membrane filter (MF) on base of funnel unit and centered evenly on the screen assembly.	1a. Funnel top removed carefully to avoid contamination. Do not place on contaminated surface. Best to hold in hand while using forceps in other.	

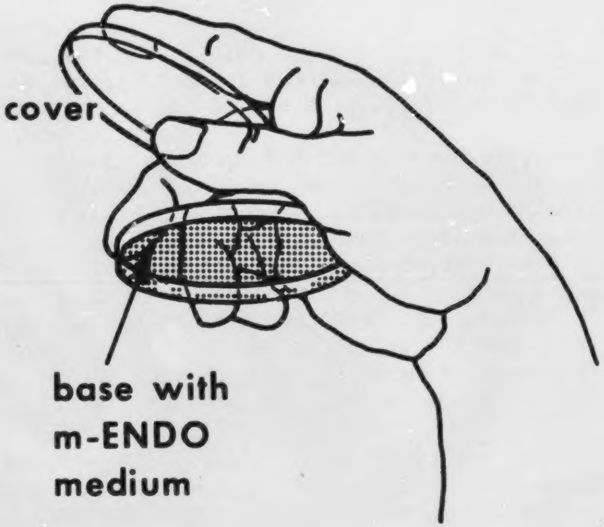
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)		1b. MF placed grid or inked side UP. MF handled with flamed forceps and only on the membranes outer 3/16 inch of its circumference. 	
		1c. Replace funnel top. Avoid over-tightening which can damage the MF or cause leakage.	
	2. Deliver a small volume of sterile buffered distilled rinse water inside the funnel.	2a. Use approximately 10 ml of water. 2b. Observe funnel for leakage. If any, disassemble unit and repeat from Step 1 after inspecting base of funnel for possible debris or damage. Persistent leakage will necessitate maintenance or replacement of funnel unit.	
	3. Deliver sample volume to funnel by using a sterile graduate.	3a. Thoroughly shake sample bottle prior to filling graduate to 40 ml mark. A minimum requirement would be 25 complete up-and-down (or back-and-forth) movements of about 0.3 ml (1 foot) in 7 seconds. 3b. Sterile graduate is prepared by oven sterilization with an aluminum foil cap.	See Flow Sheet (p. 3)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)		3c. Volume in graduate is measured by sighting if the meniscus lower curve being even with the 40 ml mark.  meniscus line 40 30 eye level glass graduate (bottom of meniscus line touching 40 ml line in a parallel plane)	
	4. Gently pour sample (40 ml) into funnel.	4a. Avoid splashing. Pour slowly and close to top of funnel without touching sides. 4b. Allow a 5 second drainage period before shaking off the last drop. 4c. Graduate marked TC (to contain): Rinse graduate several times with sterile water and pour each rinsing individually into funnel. 4d. Graduate marked TD (to deliver): Rinsing not necessary, but, allow at least 5 seconds drainage time and then gently tap off last drop into funnel.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)	5. Open vacuum control valve and remove pinch clamp to allow vacuum to filter sample through MF.	5a. Vacuum must not be allowed to enter system prior or during the previous step as this would suck sample prematurely and bacterial dispersion will not occur over membrane. 5b. Allow complete passage of sample through MF.	
	6. Rinse funnel three times with sterile buffered distilled water.	6a. Rinsings remove all of residual sample droplets from sides of funnel. 6b. Allow complete flushing of each rinse through membrane before applying next rinse. 6c. Use about 25 ml for each rinse and pass around funnel to rinse complete circumference (circular motion of hand around funnel) of funnel. Do not touch inside area of funnel.	
	7. Replace pinch clamp on suction hose.	7a. Interrupts vacuum delivery to flask. 7b. Will not allow MF to be lifted from base without possible damage due to strong suction being continued. 7c. Some laboratories may elect to use control valve for this operation and not use pinch clamp.	
	8. Disconnect funnel locking device and lift funnel from base to expose MF.	8a. Best to hold funnel in one hand while using forceps with other. Some laboratories may elect to either: *Use a germicidal cabinet to hold funnel. *Use a funnel holding device. But, in any event, DO NOT place funnel where it can become contaminated if it is to be used for another sample as is this analysis.	V.B.6.1 (p. 39)
	9. Remove membrane from funnel base.	9a. Again, handle membrane carefully with flamed forceps (quickly flamed after removing from alcohol immersion jar - NOT HEATED) and only on outer 3/16 inch of membrane.	

WATER MONITORING PROCEDURE: Total Coliform Test by the Membrane Filter Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)		9b. Break residual vacuum in flask by gently lifting edge of MF before removing.	
	10. Replace funnel on base if it is held in other hand, or, replace when convenient if held in holding device or UV light box.	10a. Funnel unit is now ready to receive the next sample as the three rinses have been found to be sufficient to cleanse the funnel of bacteria, which can influence this test (carry-over or loss of bacteria).	V.B.6.3 (p. 40)
	11. Remove cover from m-ENDO dish.	11a. Do not allow it to become contaminated. Can either be held in the hand or placed on the lab bench.	



OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued)	12. Place MF over the m-ENDO medium. Close the dish tightly when membrane shows elimination of air pockets.	12a. Grid or inked side surface contains the bacteria and must not be placed next to the m-ENDO. 12b. If air pockets persist (indicated by white areas with pink colored m-ENDO liquid) pick up the MF by its edge and re-roll. Persistent clear or white areas usually indicate that there is too little broth on the pad. Add a drop of m-ENDO to the pad if necessary while holding up a corner of the MF. Do not place broth over the membrane. 12c. Use a rolling action to eliminate air pockets. Do not run forceps or any object over the MF as it is very delicate and damage can result in poor plate results. 12d. Some amount of air spots is tolerable if they are outside the working area of where the bacteria were plated. About 3/16 inch is acceptable.	
	13. Invert petri dish (turn upside down). The bottom or plate base will now be on top and the MF will be upside down. Label dish.	13a. Use wax marker or a label used by facility. 13b. Indicate time of plating and sample number.	
	14. Place dish in the inverted position within the 35°C incubator.	14a. Plate is inverted to prevent droplets from "falling down" on MF destroying the colonial growth of the bacteria. 14b. Do not crowd plates. If a number of them have to be stacked, place them no more than three high with an unused area around them equal to the size of a petri dish. 14c. Allow an incubation period of 22-24 hours. Be sure time of plating is indicated on data sheet.	

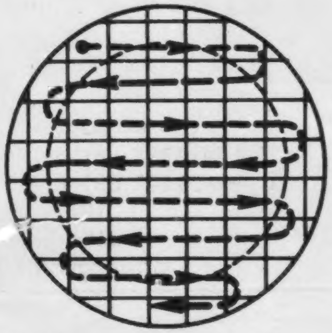
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedure (Continued) B. 8 ml Sample Volume	1. Place membrane filter (MF) on base of funnel unit and centered evenly on the screen assembly.	1a. As previously described.	
	2. Deliver a small volume of sterile buffered distilled rinse water inside the funnel.	2a. As previously described.	
	3. Deliver sample volume to funnel by using a sterile pipet.	3a. Thoroughly shake sample bottle as described previously. 3b. Fill pipet to about the 10 ml mark and apply finger pressure to hold this amount within pipet. 3c. Allow volume to fall to exactly the 8 ml graduation. Hold and maintain this volume by finger pressure.	
	4. Gently pipet the 8 ml into the funnel.	4a. As previously described in B.5A.4a-b.	
	5. Open vacuum control valve and remove pinch clamp to allow vacuum to filter sample through MF.		
	6. Rinse funnel three times with sterile buffered distilled water.	6a. As described previously.	
	7. Replace pinch clamp on suction hose.	7a. As described previously.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedures (Continued)	8. Disconnect funnel locking device and lift funnel from base to expose MF.	8a. As described previously.	
	9. Remove membrane from funnel base.	9a. As described previously.	
	10. Replace funnel on base if it is held in other hand, or, replace when convenient if held in holding device or UV light box.	10a. As described previously.	
	11. Remove cover from m-ENDO dish.	11a. As described previously.	
	12. Place MF over the m-ENDO medium. Close the dish tightly when membrane shows elimination of air pockets.	12a. As described previously.	
	13. Invert petri dish.		
	14. Place dish in the inverted position within the 35°C incubator.	14a. As described previously.	
C. 0.8 ml Sample Volume	1. Accomplish complete filtration procedure as described previously (steps 1-14) for other volumes.	1a. All items of step sequence and of this column are identical except for the means of obtaining the sample volume which is as follows: A. Use a 1 ml pipet. Fill pipet to zero mark and apply finger pressure to hold this amount within pipet. Drop level to 0.2 ml mark. B. Gently pipet the .8 ml sample volume into the funnel. Pipets may be of two general types:	

(Continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First Day Procedures (Continued)		a. TD (To Deliver)...This type allows proper volume delivery when the liquid level either drops to a marked line value or falls to the delivery tip with full finger release. b. TC (To Contain)...This type will have proper volume delivery of the full pipet contents only when the last remaining volume is forcibly ejected from the by blowing.	
D. 0.08 ml Sample Volume	1. Accomplish complete filtration procedures as described previously (steps 1-14) for other volumes.	1a. All items of step sequence and of this column are identical except for the means of obtaining the sample volume which is as follows: A. Required materials: a. Pipet, sterile, 1 ml b. Pipet, sterile, 10 ml c. 99-ml dilution blank water level adjusted, if necessary, by use of sterile pipet or sterile pipet and dilution water. B. Add 1 ml of well shaken sample water to the 99-ml blank and shake this well to distribute sample. C. Water the 10 ml pipet, obtain water from the blank and pipet 8 ml to the funnel.	
C. Second Day Procedure 1. Colony Counting Procedure	1. Remove petri dishes from incubator with careful handling to avoid jarring of plates. Turn plates over where cap is now on top.	1a. Incubation period has been within the 22-24 hour period. No deviations are permitted. 1b. Rough handling can cause spattering of droplets within plate and possibly causing difficulty in counting.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second Day Procedure (Continued)	2. Observe visually which plates are within the countable plate range (20-80 colonies). Select those within this range for counting.	2a. This ability comes with experience, but plates which are overcrowded or those with less than 20 are readily apparent. 2b. Observation of numerous colonies on the MF, even if they are not sheen containing colonies, will require counting since there is a 200 count maximum allowable colony count. The 200 colonies or more of all types (which includes coliforms) is an amount of growth which produces interferences with validity of results. 2c. It is necessary only to record counts within the given range, but, if not possible, we will cover exceptions later with examples.	
	3. Count selected plates for total coliform colonies with microscopic aid. Adjust light source to give maximum sheen development to colonies, if any.	3a. Binocular wide field dissecting microscope with 10-15x magnification preferred, but magnifying lens acceptable. 3b. Cool, white, fluorescent lighting system necessary. A near vertical light adjustment is usually optimum. 3c. Sheen is characteristic of the coliform group of bacteria and is a golden, metallic-like reflective property on the surface of the colonies. Sheen can completely or partially cover the colony. It can also appear as flecks. ANY AMOUNT OF SHEEN is considered positive.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second Day Procedure (Continued)		3d. Microscopically scan membrane with a back-and-forth movement over the grids and count all colonies having sheen.  The dashed circle indicates the effective filtering area. The dashed back-and-forth line indicates the colony counting pathway.	
		3e. Colonies are raised, usually circular, growths of original bacteria which was planted on the membrane and are considered to be the result of a single organism multiplying many times to produce a visible colony.	V.C.1.3e (p. 40)
	4. Select colony count/counts to use. Utilize formula to calculate count/100 ml.	4a. Formula $\text{Total Coliforms/100 ml} = 100 \times \frac{\text{Colony Count}}{\text{No. of mls Filtered}}$	

Example #1

mls Filtered	No. Colonies	TC/100 ml
40	TNTC (Indicates too numerous to count)	-
8	TNTC	-
0.8	TNTC	-
0.08	35	43,750

Use: 35 colonies with 0.08 ml sample volume which calculates to 44,000/100 ml (Use two significant figures).

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second Day Procedure (Continued)		Example #2	

Example #2

mls Filtered	No. Colonies	TC/100 ml
40	70	Combined calculation
8	22	
0.8	below 20	-
0.08	below 20	-

40 ml + 8 ml = 48 ml

70 colonies + 22 colonies = 92 colonies

$$\text{TC}/100\text{ ml} = 100 \times \frac{92}{48} = 190/100\text{ ml}$$

Use: 190/100 ml as count based on combined values of the plates.

Example #3

mls Filtered	No. Colonies	TC/100 ml
40	TNTC	-
8	TNTC	-
0.8	270 (80 coliforms & 190 background colonies)	>200 (greater than 200) Limit surpassed--not used
0.08	75	94,000

Use: 75 colonies from the 0.08 volume to give a count of 94,000/100 mls

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second Day Procedure (Continued)		Example #4	

Example #4

mls Filtered	No. Colonies	TC/100 ml
40	TNTC	-
8	TNTC	-
0.8	150	-
0.08	90	110,000

Rationale: Use that count which is closer to the maximum 80 count.

Example #5

mls Filtered	No. Colonies	TC/100 ml
40	17	43
8	3	-
0.8	0	-
0.08	0	-

Rationale: Use that count which is closer to the minimum 20 count.

Example #6

mls Filtered	No. Colonies	TC/100 ml
40	0	<3 (Less than 3)
8	0	-
0.8	0	-
0.08	0	-

Rationale: Assume that the largest volume delivered has one colony. Use this in calculations and call the result < (less than).

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OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Second Day Procedure (Continued)			
2. Recording Data	1. Record counts as values per 100 ml.	1a. Record and process data as required by organization/agency.	
3. Disposition of Counted Plates	1. Process to verification test, if necessary.	1a. Some, all, or none of the plates may be processed to this test dependent upon requirements of organization/agency.	V.C.3.1a
	2. Dispose of plates in approved manner.	2a. Any LIVE organisms are to be considered as POTENTIALLY DANGEROUS to humans. 2b. Usual disposition is by autoclaving (steam sterilizing) in a metal container, then discarding in waste recepticles (Note: Red dye can still cause staining so handle to preclude contamination.	

WATER MONITORING PROCEDURE: Total Coliform Test by the Membrane Filter Method

SECTION	TOPIC
I	Introduction
II*	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI*	Field and Laboratory Reagents
VII	Field and Laboratory Analyses
VIII	Safety
IX	Records and Reports

*Training guide materials are presented here under the headings marked *. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

A.10.2

Since 48 grams of m-ENDO broth powdered medium and 20 ml of 95% Ethanol are required to prepare 1 liter (1000 ml) of m-ENDO broth, it is possible to calculate weights and volumes to prepare any requirement based upon the number of plates desired. Calculations are based upon knowing the above figures and the requirement of 2.0 ml of broth for each pad saturation.

For rapid calculations the following two formulas can be used:

1. No. of plates desired x 0.096 = grams m-ENDO
2. No. of plates desired x 0.04 = ml Ethanol.

EXAMPLE: If 47 plates of m-ENDO are required:

1. 47 x 0.096 = 4.512 or 4.5 grams m-ENDO.
2. 47 x 0.04 = 1.88 or 1.9 ml Ethanol.

} 94 ml total volume

NOTE: Due to the practical and technical difficulties involved in weighing very small portions as, for instance, 0.096 grams of m-ENDO for one plate requirement, it would be wise to prepare at least 10 plates (0.96 or 1.0 gram m-ENDO and 0.4 ml Ethanol) as a minimum requirement.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.2b	A relative humidity of over 90 percent is necessary in order to obtain bacterial growth on the membrane filter (colony) which has not been inhibited by a lack of this moisture. Inhibited colonies will invariably be smaller and less apt to give the typical sheen characteristic of a frank coliform.	
	A closed container, such as a plastic vegetable crisper, may be placed within the incubator and have within the container a saturated humid atmosphere. A convenient way of accomplishing this is to wet a few paper towels and place within the crisper or box.	
	An accurate solid heat sink incubator is acceptable for use. This is constructed of a solid metallic block having slots for insertion of the petri dishes. Since there are no provisions for a high humidity chamber in this type of incubator, it is important to only use the types of petri dishes having a tight attachment of cover-to-base thus preventing loss of moisture during the incubation period.	Std. Meth. 14:937

TRAINING GUIDE NOTE

REFERENCES/RESOURCES

A.1.1

Incubator should be kept out of drafts or direct sunlight in order to prevent temperature inside the incubator from changing outside the temperature range specified (35°C ± 0.5°C).

Power supply should be selected so that there will not be too many pieces of equipment on the same circuit. Otherwise circuits will be blown repeatedly.

Standard Methods for the Examination of Water and Wastewater, 14th Ed. (1975) APHA, WPCF, AWWA, p. 880 ff. (Hereafter referred to as: Std. Meth. 14:(Page No.)

A.1.3

Mercury bulb thermometer usually used in most incubators and a recording thermometer are acceptable. Thermometers must be calibrated against a mercury bulb thermometer which is (or calibrated against) a National Bureau of Standards issue and used with the certificate and correction chart.

A.2

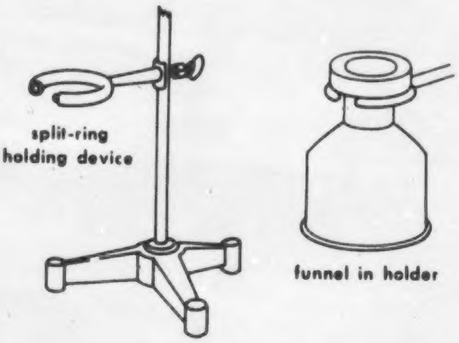
Sterilizing ovens should be of sufficient size to prevent crowding of materials to be sterilized. The information below summarizes the use of the oven.

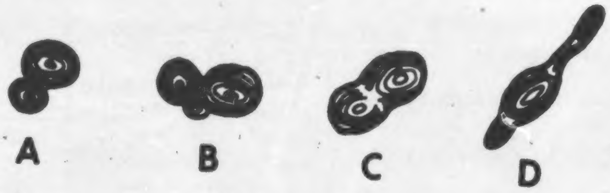
MATERIAL STERILIZED	CONDITIONS	REMARKS
Glassware	170°C for at least 60 min.	If internal oven, characteristics are unknown
Glassware	160°C for at least 60 min.	If oven temperature uniform throughout chamber
Glassware within metal container	170°C for at least 120 min.	
Other material	170°C for at least 60 min.	Material must be capable of withstanding sterilizing conditions

Alternately, a gas sterilizing unit can be used in place of the hot-air oven. Refer to Standard Methods and manufacturer's catalogs for details of such a unit (ethylene oxide gas).

Conflicting temperature/time relationships appear in differing references, but, the over-riding consideration is how this time/temperature relationship works in your hands, with your equipment, and considering the results of sterility testing.

Std. Meth. 14:881
:885

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.4	Distilled water must not contain substances preventing bacterial growth or be highly nutritive. There are required procedures for testing distilled water and should be undertaken only by professional bacteriologists or in laboratories where this is done regularly. Alternately, a source of deionized water which meets all requirements as imposed on distilled water is suitable for use in bacteriological operations.	Std. Meth. 14:887
B.4.2	Diagram and equipment listing describe the type of units most commonly used in the membrane filter procedure. They are by no means the only acceptable arrangement which can be utilized. Different modes of obtaining a vacuum or its control are available. Also, various vacuum flask arrangements are acceptable as well as the types of vacuum controls. To preclude numerous examples, the one described will be sufficient to give technological procedures which are acceptable and the reader can refer to Standard Methods and Manufacturer's catalogs for further information regarding system components or field units which are acceptable.	
B.6.1	A germicidal cabinet is an enclosed unit which contains an active germicidal lamp (UV) which produces a 99.9% bacterial kill in 2 minutes. It is important not to have UV leakage from cabinet which can be detrimental to the analyst's eyes. A funnel holding device is designed to hold the funnel and prevent its contamination. EXAMPLE 	Std. Meth. 14:933

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.6.3	Funnel units are considered to be acceptable for use for the next sample unless an interval of 30 minutes or longer elapses before the next sample is run. In this case the unit must be resterilized.	Std. Meth. 14:932
C.1.3e	Occasionally colonial growth will be observed to be irregular such as the following:  Usually, as in A and B, the colonies are readily discernible as being multiple colonies - 2 for A and 3 for B. In the case of C and D, however, this separation is not readily apparent and the judgment, based on experience, of the analyst becomes important. In the case of D the long strand growth may be caused by a particle of debris which allowed channeled growth of one or more bacteria.	
C.3.1a	The verification test is accomplished by picking the presumptive sheened coliform colony with a sterile needle and passing it through a series of broth media to observe for another coliform characteristic-gas formation in a selective medium. Refer to Standard Methods for a detailed performance of this verification test.	Std. Meth. 14:920 :931

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

A.10.1 Procedures are given for m-ENDO broth medium preparation which is, however, not the only acceptable method available. Other acceptable m-ENDO medium preparations include:

A. m-ENDO Agar Medium — Std. Meth. 14:895

This includes the addition of the complex carbohydrate agar whose purpose is to solidify the medium. In this preparation the absorption pad is not required for the analysis.

B. Pre-prepared Ampouled m-ENDO Medium

A complete prepared medium which is enclosed in a glass tube. Contains enough medium for a single test and has the advantages of a longer shelf life and convenience of use. Is somewhat more costly than laboratory preparation, especially when many plates are to be processed.

A.10.4 Ethanol is added to distilled water in a 2% dilution for the m-ENDO medium. The amounts, of course, would be different depending on the petri dish requirements. The table below gives some useful information as reference:

No. of Plates m-ENDO Required	Alcohol-Water Required, mls	Ethanol mls	m-ENDO Broth Powder, gm
500	1 liter (1000 ml)	20	48
250	500	10	24
50	100	2	4.8
25	50	1	2.4
10	20	0.4	.96 or 1.0
5	10	0.2	.48 or .5

Some laboratories prepare a large amount of the 2% solution and, when tightly stoppered, can be used for extended periods.

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LABORATORY SAFETY PRACTICES

I INTRODUCTION

A Safe Use, Handling and Storage of Chemicals

1 Chemicals in any form can be safely stored, handled, and used if their hazardous physical and chemical properties are fully understood and the necessary precautions, including the use of proper safeguards and personal protective equipment are observed.

2 The management of every unit within a manufacturing establishment must give wholehearted support to a well integrated safety policy.

B General Rules for Laboratory Safety

1 Supervisory personnel should think "safety." Their attitude toward fire and safety standard practices is reflected in the behavior of their entire staff.

2 A safety program is only as strong as the worker's will to do the correct things at the right time.

3 The fundamental weakness of most safety programs lies in too much lip service to safety rules and not enough action in putting them into practice.

4 Safety practices should be practical and enforceable.

5 Accident prevention is based on certain common standards of education, training of personnel and provision of safeguards against accidents.

II LABORATORY DESIGN AND EQUIPMENT

A Type of Construction

1 Fire-resistant or noncombustible

2 Multiple story buildings should have adequate means of exit.

3 Stairways enclosed with brick or concrete walls

4 Laboratories should have adequate exit doors to permit quick, safe escape in an emergency and to protect the occupants from fires or accidents in adjoining rooms. Each room should be checked to make sure there is no chance of a person being trapped by fire, explosions, or release of dangerous gases.

5 Laboratory rooms in which most of the work is carried out with flammable liquids or gases should be provided with explosion-venting windows.

B Arrangement of Furniture and Equipment

1 Furniture should be arranged for maximum utilization of available space and should provide working conditions that are efficient and safe.

2 Aisles between benches should be at least 4 feet wide to provide adequate room for passage of personnel and equipment.

3 Desks should be isolated from benches or adequately protected.

4 Every laboratory should have an eye-wash station and a safety shower.

C Hoods and Ventilation

1 Adequate hood facilities should be installed where work with highly toxic or highly flammable materials are used.

2 Hoods should be ventilated separately and the exhaust should be terminated at a safe distance from the building.

3 Make-up air should be supplied to rooms or to hoods to replace the quantity of air exhausted through the hoods.

4 Hood ventilation systems are best designed to have an air flow of not less than 60 linear feet per minute across the face of the hood, with all doors open and 150, if toxic materials are involved.

5 Exhaust fans should be spark-proof if exhausting flammable vapors and corrosive resistant if handling corrosive fumes.

6 Controls for all services should be located at the front of the hood and should be operable when the hood door is closed.

7 All laboratory rooms should have the air changed continuously at a rate depending on the materials being handled.

D Electrical Services

1 Electrical outlets should be placed outside of hoods to afford easy access and thus protect them from spills and corrosion by gases.

2 Noninterchangeable plugs should be provided for multiple electrical services.

3 Adequate outlets should be provided and should be of the three-pole type to provide for adequate grounding.

E Storage

1 Laboratories should provide for adequate storage space for mechanical equipment and glassware which will be used regularly.

2 Flammable solvents should not be stored in glass bottles over one liter in size. Large quantities should be stored in metal safety cans. Quantities requiring containers larger than one gallon should be stored outside the laboratory.

3 Explosion proof refrigerators should be used for the storage of highly volatile and flammable solvents.

4 Cylinders of compressed or liquified gases should not be stored in the laboratory.

F Housekeeping

1 Housekeeping plays an important role in reducing the frequency of laboratory accidents. Rooms should be kept in a neat orderly condition. Floors, shelves, and tables should be kept free from dirt and from all apparatus and chemicals not in use.

2 A cluttered laboratory is a dangerous place to work. Maintenance of a clean and orderly work space is indicative of interest, personal pride, and safety-mindedness.

3 Passageways should be kept clear to all building exits and stairways.

4 Metal containers should be provided for the disposal of broken glassware and should be properly labeled.

5 Separate approved waste disposal cans, should be provided for the disposal of waste chemicals.

6 Flammable liquids not miscible with water and corrosive materials, or compounds which are likely to give off toxic vapors should never be poured into the sink.

G Fire Protection

1 Laboratory personnel should be adequately trained regarding pertinent fire hazards associated with their work.

2 Personnel should know rules of fire prevention and methods of combating fires.

3 Fire extinguishers (CO_2 type) should be provided at convenient locations and personnel should be instructed in their use.

4 Automatic sprinkler systems are effective for the control of fires in chemical laboratories.

H Alarms

1 An approved fire alarm system should be provided.

2 Wherever a hazard of accidental release of toxic gases exists, a gas alarm system to warn occupants to evacuate the building should be provided.

3 Gas masks of oxygen or compressed air type should be located near exits and selected personnel trained to use them.

III HANDLING GLASSWARE

A Receiving, Inspection and Storage

1 Packages containing glassware should be opened and inspected for cracked or nicked pieces, pieces with flaws that may become cracked in use, and badly shaped pieces.

2 Glassware should be stored on well-lighted stockroom shelves designed and having a coping of sufficient height around the edges to prevent the pieces from falling off.

B Laboratory Practice

1 Select glassware that is designed for the type of work planned.

2 To cut glass tubing or a rod, make a straight clean cut with a cutter or file at the point where the piece is to be severed. Place a towel over the piece to protect the hands and fingers, then break away from the body.

3 Large size tubing is cut by means of a heated nichrome wire looped around the piece at the point of severance.

4 When it is necessary to insert a piece of glass tubing or a rod through a perforated rubber or cork stopper, select the correct bore so that the insertion can be made without excessive strain.

5 Use electric mantels for heating distillation apparatus, etc.

6 To remove glass splinters, use a whisk broom and a dustpan. Very small pieces can be picked up with a large piece of wet cotton.

IV GASES AND FLAMMABLE SOLVENTS

A Gas Cylinders

1 Large cylinders must be securely fastened so that they cannot be dislodged or tipped in any direction.

2 Connections, gauges, regulators or fittings used with other cylinders must not be interchanged with oxygen cylinder fittings because of the possibility of fire or explosion from a reaction between oxygen and residual oil in the fitting.

3 Return empty cylinders promptly with protective caps replaced.

B Flammable Solvents

1 Store in designated areas well ventilated.

2 Flash point of a liquid is the temperature at which it gives off vapor sufficient to form an ignitible mixture with the air near the surface of the liquid or within the vessel used.

3 Ignition temperature of a substance is the minimum temperature required to initiate or cause self-sustained combustion independently of the heating or heated element.

4 Explosive or flammable limits. For most flammable liquids, gases and solids there is a minimum concentration of vapor in air or oxygen below which propagation of flame does not occur on contact with a source of ignition. There is also a maximum proportion of vapor or gas in air above which

propagation of flame does not occur. These limit mixtures of vapor or gas with air, which if ignited will just propagate flame, are known as the "lower and higher explosive or flammable limits."

5 Explosive Range. The difference between the lower and higher explosive or flammable limits, expressed in terms of percentage of vapor or gas in air by volume is known as the "explosive range."

6 Vapor Density is the relative density of the vapor as compared with air.

7 Underwriter's Laboratories Classification is a standard classification for grading the relative hazard of the various flammable liquids. This classification is based on the following scale:

Ether Class	100
Gasoline Class.........	90 - 100
Alcohol (ethyl) Class....	60 - 70
Kerosene Class	30 - 40
Paraffin Oil Class	10 - 20

8 Extinguishing agents

V CHEMICAL HAZARDS

A Acids and Alkalies

1 Some of the most hazardous chemicals are the "strong" or "mineral" acids such as hydrochloric, hydrofluoric, sulfuric and nitric.

2 Organic acids are less hazardous because of their comparatively low ionization potentials. However, such acids as phenol (carbolic acid), hydrocyanic and oxalic are extremely hazardous because of their toxic properties.

3 Classification of acids

B Oxidizing Materials

1 Such oxidizing agents as chlorates, peroxides, perchlorates and perchloric acid, in contact with organic matter can cause explosions and fire.

2 They are exothermic and decompose rapidly, liberating oxygen which reacts with organic compounds.

3 Typical hazardous oxidizing agents are:

Chlorine Dioxide
Sodium Chlorate
Potassium Chromate
Chromium Trioxide
Perchloric Acid

C Explosive Power

1 Many chemicals are explosive or form compounds that are explosive and should be treated accordingly.

2 A few of the more common examples of this class of hazardous materials are:

Acetylides
Silver Fulminate
Peroxides
Peracetic Acid
Nitroglycerine
Picric Acid
Chlorine and Ethylene
Sodium Metal
Calcium Carbide

D Toxicity

1 Laboratory chemicals improperly stored or handled can cause injury to personnel by virtue of their toxicity.

2 Types of exposure. There are four types of exposure to chemicals:

a Contact with the skin and eyes

b Inhalation

c Swallowing

d Injection

VI PRECAUTIONARY MEASURES

A Clothing and Personal Protective Equipment

1 Chemical laboratories should have special protective clothing and equipment readily available for emergency use and for secondary protection of personnel working with hazardous materials.

2 Equipment should be provided for adequate:

- a Eye protection
- b Body protection
- c Respiratory protection
- d Foot protection
- e Hand protection

B Bodily Injury

1 Burns, eye injuries, and poisoning are the injuries with which laboratory people must be most concerned.

2 First emphasis in the laboratory should be on preventing accidents. This means observing all recognized safe practices using necessary personal protective equipment and exercising proper control over poisonous substances at the source of exposure.

3 So that a physician can be summoned promptly, every laboratory should have posted the names, telephone numbers, and addresses of doctors to be called in an emergency requiring medical care.

REFERENCES

Guide for Safety in the Chemical Laboratory, the General Safety Committee of the Manufacturing Chemists Association, Inc., Van Nostrand, New York (1954).

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